



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>G01N 33/53</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 95/28640</b> <b>(43) International Publication Date:</b> 26 October 1995 (26.10.95)
<b>(21) International Application Number:</b> PCT/US95/04683 <b>(22) International Filing Date:</b> 13 April 1995 (13.04.95)  <b>(30) Priority Data:</b> 08/227,007 13 April 1994 (13.04.94) US  <b>(60) Parent Application or Grant</b> (63) Related by Continuation US 08/227,007 (CIP) Filed on 13 April 1994 (13.04.94)  <b>(71) Applicants (for all designated States except US):</b> THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK [US/US]; West 116th Street & Broadway, New York, NY 10027 (US). COLD SPRING HARBOR LABORATORY [US/US]; One Bungtown Road, Cold Spring Harbor, NY 11724 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> STILL, W., Clark [US/US]; Fiddlers Bridge Road at Lake Drive, Clinton, NY 12514 (US). WIGLER, Michael, H. [US/US]; One Walden Court, Lloyd Harbor, NY 11743 (US). OHLMEYER, Michael, H., J. [US/US]; 903 Deer Creek Drive, Plainsboro,		NJ 08536 (US). DILLARD, Lawrence, W. [US/US]; 38-06 Fox Run Drive, Plainsboro, NJ 08536 (US). READER, John, C. [GB/US]; 112 Biscayne Court #8, Princeton, NJ 08540 (US).  <b>(74) Agent:</b> WHITE, John, P.; Cooper & Dunham L.L.P, 1185 Avenue of the Americas, New York, NY 10036 (US).  <b>(81) Designated States:</b> AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).  <b>Published</b> With international search report.
<b>(54) Title:</b> COMPLEX COMBINATORIAL CHEMICAL LIBRARIES ENCODED WITH TAGS		
<b>(57) Abstract</b>  Encoded combinatorial chemistry is provided, where sequential synthetic schemes are recorded using organic molecules, which define choice of reactant, and stage, as the same or different bit of information. Various products can be produced in the multi-stage synthesis, such as oligomers and synthetic non-repetitive organic molecules. Conveniently, nested families of compounds can be employed as identifiers, where number and/or position of a substituent define the choice. Alternatively, detectable functionalities may be employed, such as radioisotopes, fluorescers, halogens, and the like, where presence and ratios of two different groups can be used to define stage or choice. Particularly, pluralities of identifiers may be used to provide a binary or higher code, so as to define a plurality of choices with only a few detachable tags. The particles may be screened for a characteristic of interest, particularly binding affinity, where the products may be detached from the particle or retained on the particle. The reaction history of the particles which are positive for the characteristic can be determined by the release of the tags and analysis to define the reaction history of the particle.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

**COMPLEX COMBINATORIAL CHEMICAL LIBRARIES ENCODED WITH TAGS**

This application is a continuation-in-part of U.S. Serial No. 08/227,007, filed April 13, 1994 the contents of which are hereby incorporated by reference into the subject application.

5

IntroductionTechnical Field

The field of this invention concerns combinatorial chemistry which involves syntheses having a plurality of stages, with each stage involving a plurality of choices, where large numbers of products having varying compositions are obtained.

Background of the Invention

There is substantial interest in devising facile methods for the synthesis of large numbers of diverse compounds which can then be screened for various possible physiological or other activities. Typically such a synthesis involves successive stages, each of which involves a chemical modification of the then existing molecule. For example, the chemical modification may involve the addition of a unit, e.g. a monomer or synthon, to a growing sequence or modification of a functional group. By employing syntheses where the chemical modification involves the addition of units, such as amino acids, nucleotides, sugars, lipids, or heterocyclic compounds where the units may be naturally-occurring, synthetic, or combinations thereof, one may create a large number of compounds. Thus, even if one restricted the synthesis to naturally-occurring units or building blocks, the number of choices would be very large, 4 in the case

-2-

of nucleotides, 20 in the case of the common amino acids, and essentially an unlimited number in the case of sugars.

5 One disadvantage heretofore inherent in the production of large numbers of diverse compounds, where at each stage of the synthesis there are a significant number of choices, is the fact that each individual compound will be present in a minute amount. While a characteristic of a particular compound, e.g. a physiological activity, may be  
10 determinable, it is usually impossible to identify the chemical structure of the particular compound present.

Moreover, physiologically-active compounds have historically been discovered by assaying crude broths  
15 using Edisonian or stochastic techniques, where only a relatively few compounds are assayed at a time, or where a limited number of structurally similar homologs of naturally-occurring physiologically-active compounds are assayed. Two major problems have been associated with the  
20 use of such crude broths, namely, the necessity to purify the reaction mixture into individual component compounds and the time-consuming effort required to establish the structure of the compound once purified.

25 To address these disadvantages and problems, techniques have been developed in which one adds individual units as part of a chemical synthesis sequentially, either in a controlled or a random manner, to produce all or a substantial proportion of the possible compounds which can  
30 result from the different choices possible at each sequential stage in the synthesis. However, for these techniques to be successful it is necessary for the compounds made by them to be amenable to methods which will allow one to determine the composition of a  
35 particular compound so made which shows a characteristic of interest.



-3-

One such approach involves using a chip which allows for separate analysis at physically separate sites on the surface of the chip (Fodor et al., *Science* 251: 767 [1991]). By knowing which reactant is added sequentially at each such site, one can record the sequence of events and thus the series of reactions. If one then subjects the chip to a screening method for a particular desired characteristic and detects the characteristic one can readily determine the compound synthesized at the site which demonstrates that characteristic.

Another such technique involves the theoretical synthesis of oligonucleotides in parallel with the synthesis of oligopeptides as the compounds of interest (Brenner and Lerner, *PNAS USA* [1992] 81: 5381-5383).

Further techniques are also disclosed in the following publications: Amoto, *Science* (1992) 257, 330-331, discusses the use of cosynthesized DNA labels to identify polypeptides. Lam, et al., *Nature* (1991) 354, 82-84, describe a method for making large peptide libraries. Houghton, et al., *Nature* (1991) 354, 84-86, and Jung and Beck-Sickinger, *Angew. Chem. Int. Ed. Engl.* (1992) 91, 367-383, describe methodology for making large peptide libraries. Kerr et al., *J. Amer. Chem. Soc.*, (1993) 115, 2529-31, teach a method of synthesizing oligomer libraries encoded by peptide chains. Finally, international applications WO 91/17823 and WO 92/09300 concern combinatorial libraries.

However, since methods such as the preceding typically require the addition of like moieties, there is substantial interest in discovering methods for producing compounds which are not limited to sequential addition of like moieties. Such methods would find application, for example, in the modification of steroids, antibiotics, sugars, coenzymes, enzyme inhibitors, ligands and the

-4-

like, which frequently involve a multi-stage synthesis in which one would wish to vary the reagents and/or conditions to provide a variety of compounds. In such methods the reagents may be organic or inorganic reagents, where functionalities may be introduced or modified, side groups attached or removed, rings opened or closed, stereochemistry changed, and the like. (See, for example, Bunin and Ellman, *JACS* 114, 10997 [1992].) For such a method to be viable, however, there needs to be a convenient way to identify the structures of the large number of compounds which result from a wide variety of different modifications. Thus, there is a need to find a way whereby the reaction history may be recorded, and desirably, the structures of the resultant compounds identified.

Finally, as the size of a library of compounds so synthesized increases, known techniques of structure elucidation and product segregation introduce substantial inefficiencies and uncertainties which hinder the accurate determination of the structure of any compound identified as being of interest. Thus, there is a substantial need for new methods which will permit the synthesis of complex combinatorial chemical libraries which readily permit accurate structural determination of individual compounds within the library which are identified as being of interest.

Many of the disadvantages of the previously-described methods as well as many of the needs not met by them are addressed by the present invention which, as described more fully hereinafter, provides myriad advantages over these previously-described methods.

Summary of The Invention

Methods and compositions are provided for encoded combinatorial libraries, whereby at each stage of the synthesis, a support, such as a particle, upon which a compound is being synthesized is uniquely tagged to define a particular event, usually chemical, associated with the synthesis of the compound on the support. The tagging is accomplished using identifier molecules which record the sequential events to which the supporting particle is exposed during synthesis, thus providing a reaction history for the compound produced on the support.

Each identifier molecule is characterized by being stable under the synthetic conditions employed, by remaining associated with the supports during the synthesis, by uniquely defining a particular event during the synthesis which reflects a particular reaction choice at a given stage of the synthesis, by being distinguishable from other components that may be present during assaying, and by allowing for detachment of a tag component which is discernible by a convenient, analytical technique.

The identifiers of this invention are used in combination with one another to form a binary or higher order encoding system permitting a relatively small number of identifiers to be used to encode a relatively large number of reaction products. For example, when used in a binary code  $N$  identifiers can uniquely encode up to  $2^N$  different compounds.

Moreover, the identifiers of this invention need not be bound serially through a previous identifier but rather are individually bound to the substrate, either directly or through the product being synthesized. The identifiers are not sequencable. Furthermore, the identifiers contain a cleavable member or moiety which permits detachment of a tag component which can be readily analyzed.

- 6 -

Conveniently, the combinatorial synthesis employs definable solid supports upon which reactions are performed and to which the identifiers are bound. The individual solid supports or substrates carrying the final product compounds may be screened for a characteristic of interest and the reaction history determined by analyzing the associated identifier tags.

5

-7-

Brief Description of the Drawings

- Figure 1 illustrates the analysis of tag 4 by mass spectroscopy. Two signals corresponding to tag 4 were observed.
- 5 Figure 2 illustrates the analysis of tag 11 by mass spectroscopy. Two signals corresponding to tag 11 were observed.
- 10 Figure 3 illustrates the analysis of tag 13 by mass spectroscopy. Two signals corresponding to tag 13 were observed.
- 15 Figure 4 illustrates the analysis of tags 4, 11 and 13 by positive chemical ionization mass spectroscopy (PCIMS) when approximately equal amounts of each tag were mixed together. Two signals corresponding to each separate tag could easily be distinguished.
- 20 Figure 5 illustrates the mass spectrum and chromatogram for the underivatized d-7 sample only.
- 25 Figure 6 illustrates the mass spectra and chromatograms showing the improvement in chromatography due to derivatization of tags, a d-5 and d-7 mixed sample are assessed.

DETAILED DESCRIPTION OF THE INVENTION

As used in this application the term "tag" or "T" means a chemical moiety which possesses two properties. First, it is capable of being distinguished from all other chemical moieties. Second, it is capable of being detected when present at  $10^{-18}$  to  $10^{-9}$  mole. These two properties may be embodied in a single chemical structure. Alternatively, these properties may be embodied in separate chemical structures which are linked together. In this latter case, one of the chemical structures, which may be designated  $\underline{C}$  (or in the case of more than one such structure  $\underline{C}$ ,  $\underline{C}'$ , etc.) provides the property of rendering the tag distinguishable from other tags while the other chemical structure, E, provides the property of rendering the tag detectable and optionally may provide the property of rendering the tag separable from other tags.

As used in this application, the term "linker" or "L" means a chemical moiety which possesses three properties. First, it is attachable to a solid support. Second, it is attachable to a tag. Third, when it is attached to both a solid support and a tag, it is cleavable such that the tag may be released from the solid support. These three properties may be embodied in a single chemical structure. Alternatively, these properties are embodied in three chemical structures which are linked together. In this latter case one of the chemical structures, which may be designated  $F^1$ , provides the property of rendering the linker attachable to the solid support; the second chemical structure, which may be designated V, provides the property of rendering the linker cleavable; and the third chemical structure which may be designed  $A'$ , provides the property of rendering the linker attachable to the tag. Desirably, the chemical structures V and  $A'$  are one and the same, in which case V- $A'$  may be designated  $F^2$ .

-9-

As used in this application, the term "identifier" means a chemical entity which includes both a tag and a linker. Thus, in the broadest sense an identifier may be represented by the formula L-T while specific embodiments of the identifier may be represented by the formulae F<sup>1</sup>-V-A'-T; F<sup>1</sup>-V-A'-C-E (or F<sup>1</sup>-V-A'-E-C); L-C-E (or L-E-C); and L-C-E-C'.

As used in this application, the term "bound identifier" means an identifier attached to a solid support.

As used herein, the term "choice" means the alternative variables for a given stage in a combinatorial synthesis, such as reactant, reagent, reaction conditions, and combinations thereof. The term "stage" corresponds to a step in the sequential synthesis of a compound or ligand; the compound or ligand being the final product of a combinatorial synthesis.

The term "alkyl" includes linear, branched, and cyclic structures and combinations thereof. Thus, the term includes methyl, ethyl, propyl, isopropyl, butyl, sec- and tert-butyl, cyclopropyl, cyclobutyl, cyclopentyl, 2-methylcyclopropyl, and the like. Lower alkyl is C<sub>1</sub>-C<sub>6</sub> alkyl. Lower alkenyl is C<sub>2</sub>-C<sub>6</sub> alkenyl of a linear, branched, or cyclic configuration and combinations thereof.

Unless otherwise indicated, it is intended that the definitions of any substituent (e.g., R<sup>1</sup> R<sup>2</sup>, Z, etc.) in a particular molecule be independent of its definitions elsewhere in the molecule. Thus, NR<sup>4</sup>R<sup>4</sup> represents NHH, NHCH<sub>3</sub>, NHCH<sub>2</sub>CH<sub>3</sub>, N(CH<sub>3</sub>)<sub>2</sub>, etc.

Some of the compounds described herein contain one or more centers of asymmetry and may thus give rise to enantiomers, diastereoisomers, and other stereoisomeric

-10-

forms. The present invention is meant to include all such possible stereoisomers as well as their racemic and optically pure forms. Optically active (R) and (S) isomers may be prepared using chiral synthons, chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic double bonds, it is intended to include both E and Z geometric isomers.

The materials upon which the combinatorial syntheses of this invention are performed are referred to herein interchangeably as beads, solid surfaces, (solid) substrates, particles, supports, etc. These terms are intended to include:

- a) solid supports such as beads, pellets, disks, capillaries, hollow fibers, needles, solid fibers, cellulose beads, pore-glass beads, silica gels, polystyrene beads optionally cross-linked with divinylbenzene, grafted co-poly beads, poly-acrylamide beads, latex beads, dimethylacrylamide beads optionally cross-linked with N,N'-bis-acryloyl ethylenediamine, glass particles coated with a hydrophobic polymer, etc., i.e., a material having a rigid or semi-rigid surface; and
- b) soluble supports such as low molecular weight non-cross-linked polystyrene.

These materials must contain functionalities or must be able to be functionalized such that identifiers or product intermediates may be attached to them.

In addition, the following abbreviations have the indicated meanings:

AcOH	=	acetic acid
BSA	=	bis(trimethylsilyl)acetamide
CAN	=	cerium (iv) ammonium nitrate



-11-

	DEAD	=	diethyl azodicarboxylate
	DCM	=	dichloromethane
	DIC	=	diisopropylcarbodiimide
	DMF	=	N,N-dimethylformamide
5	Fmoc	=	9-fluorenylmethoxycarbonyl
	HOBt	=	1-hydroxybenzotriazole
	PhMe	=	toluene
	r.t.	=	room temperature
	TFA	=	trifluoroacetic acid
10	THF	=	tetrahydrofuran

The subject invention concerns the production of libraries of products, i.e. compounds, where the individual products or compounds present in the libraries may be physically separated from one another and may be screened for a characteristic of interest either bound to, or detached from, a solid support. By having serial syntheses, where at each stage of a synthesis each of the individual intermediates is treated in a variety of ways, a very large number of products is produced, each of which is present in a small amount, frequently less than 100 pmol, more frequently less than 10 nmol. Because of the small quantity of final product or compound so produced, identifying these products by isolating and structurally elucidating the products would generally not be feasible. Moreover, in sequential synthesis involving other than the addition of similar units, the analysis would be arduous if not impossible using the amount of product typically available. However, by associating each choice or combination of choices (e.g., "add reagent A" or "add reagent A, then reagent B, and heat to 100°C for 2 hrs.") of the serial synthesis with a combination of identifiers which define the choice of variables such as reactant, reagent, reaction conditions, or a combination of these, one can use the identifiers to define the reaction history of each definable and separable substrate. The analysis of tags detached from the identifiers allows for ready

-12-

identification of the reaction history, at picomolar or lower concentrations, e.g. femtomolar or less. One can determine a characteristic of a product of a synthesis, usually a chemical or biological characteristic by various screening techniques, and then identify the reaction history and thereby the structure of that product, which has the desired characteristic, by virtue of the tags associated with the product.

10 The use of the instant multiple tag system avoids the necessity of carrying out a complicated cosynthesis which reduces yields and requires multiple protecting groups, and avoids the necessity of using sequencable tags which are necessarily chemically labile. Both the necessity of  
15 multiple protecting groups and the intrinsic instability of all known sequencable tagging molecules (i.e., nucleic acid or peptide oligomers) severely limit the chemistry which may be used in the synthesis of the library element or ligand.

20 Moreover, the use of a binary, or higher order, multiple tag system reduces enormously the number of tags necessary to encode the reagent/reactant choice in any stage in a synthesis. For example, if a particular synthetic stage  
25 could be carried with 128 different choices for reagent, the binary system would require only 7 tags. This can make the difference between a practical encoding system and an impractical one, because it may not be feasible to obtain and use the large number of distinguishable tags  
30 required by other systems. With the binary system of the invention, 30 distinguishable tags are available and are sufficient to encode  $>10^9$  different syntheses.

35 Importantly, the present method employs tags which are detachable from a ligand or solid support for the purpose of decoding. Such detachability also allows the tags to be distinguished on more than one basis; in particular,

-13-

they can be separated (e.g., on the basis of chromatographic retention time) and then analyzed (e.g., a second basis is a spectral property such as mass spectroscopy m/e, or electrophoricity). Having multiple  
5 bases for distinction allows the encoding of large amounts of information with a small number of tags.

Detachment further allows tags to be detected at very low levels, because they can be removed from the support  
10 matrix on which the synthesis is effected and from the ligand synthesized, the presence of either of which could provide spurious background signals, e.g. by quenching fluorescence or the like.

15 Detachable tags are also amenable to rapid analysis by automated sampling systems, and allow for selective derivatization for detection via functional groups, eliminating any incompatibility between the detection moiety and the reaction conditions used in the synthesis.

20 Inherent in any tagging scheme is the requirement that the chemical characteristics of the tags and the chemical stages for their incorporation be compatible with the characteristics of the ligand and the stages in their  
25 synthesis, and vice versa. The advantage of tags that are generally unreactive, as exemplified hereinafter by the substituted-aryloxypolymethylene moieties, is a greater range of chemical transformations and chemical functionality that can be employed in synthesis of the  
30 ligands.

A further advantage of the chemically stable tags of this invention is their compatibility with a greater variety of rapid, convenient methods of separation and analysis, such  
35 as gas chromatography and mass spectrometry. Moreover, the organic tags of these invention generally do not specifically interact with biological receptors. Thus,

-14-

these tags will generally not give spurious results in biological assays and will generally not be modified by enzymes or other biological molecules.

- 5 Finally, the chemical stability of the present tags allows them to be detached by a wide variety of methods which improves sensitivity in their analysis as described above.

10 Thus, this invention provides methods and compositions for encoded combinatorial synthesis whereby at each stage of the synthesis one or more identifiers are provided which encode an event associated with a particle stage in the synthesis of a compound on a support or particle. This event comprises the choice of reactant and/or reaction  
15 conditions at that stage of the reactions where each such stage may involve one or more reactants which are the same or different under the same or different conditions, e.g. partial reactions, multiple additions, rate of addition, differing combinations of reagents, etc. In addition,  
20 groups of particles may be sequestered from other groups of particles and subjected to a different series of events at any time during the course of the sequential synthesis.

By providing N identifiers, each having M distinguishable  
25 states,  $M^N$  different syntheses can be uniquely defined. In the case of  $M=2$  where the two states could be the presence or absence of identifier, the synthesis would thus be defined by a base 2 or binary code. In the case of  $M=3$  where the three states could be the presence of an  
30 identifier at two distinguishable concentrations or its absence, the synthesis would be defined by a base 3 code. Herein, such base M codes where  $M>2$  are termed higher order codes. The advantage of higher order codes over a binary code is that fewer identifiers are required to  
35 encode the same quantity of information about the synthesis. The products which are produced will be defined as resulting from a serial synthesis. At each

-15-

stage in the synthesis, there is available a plurality of reactants and/or reagents and/or conditions, which result in a feature of the product in relation to an identifiable and usually separable entity, e.g. tag. In referring to reactants and reagents, it is intended that the reactant, for the most part, becomes incorporated into the product, e.g. an amino acid, nucleotide, nucleophile, electrophile, diene, alkylating or acylating agent, diamine, or any other synthon, etc. while a reagent may or may not become incorporated into the product, e.g. base, acid, heat, oxidizing or reducing agent, while both will be included under the term "agent". The synthesis may involve individual reactants which become incorporated into the product. Alternatively, a stage may involve one or more reactions which result in a modification of a reaction intermediate. In many cases, combinations of these possibilities will be involved.

Using a base 2 or binary code ( $M=2$ ) and three identifiers ( $N=3$ ), as many as 8 ( $2^3$ ) agents for a given stage in a synthesis may be encoded. If the three identifiers are represented as T1, T2, and T3 and the presence or absence of each identifier is represented as a '0' or '1' respectively, then eight different agents could be represented in a binary code as follows:

	Agent 1	Agent 2	Agent 3	Agent 4
T1,T2,T3	0,0,0	1,0,0	0,1,0	1,1,0
	Agent 5	Agent 6	Agent 7	Agent 8
T1,T2,T3	0,0,1	1,0,1	0,1,1	1,1,1

Similarly, even more information about the synthesis may be encoded by more identifiers. For example, 9 identifiers ( $N=3$ ) and a base 2 code ( $M=2$ ) would allow up to  $2^9$  or 512 different agent choices to be encoded. Using a base 3 code ( $M=3$ ) and three identifiers ( $N=3$ ) would

-16-

allow as many as 27 ( $3^3$ ) agent choices to be encoded. If the three identifiers are represented as T1, T2 and T3, and the absence of an identifier is represented as a '0', its presence at a quantity of ~0.5 pmol/bead as a '1', and its presence at a quantity at ~1.0 pmol/bead as a '2', then the 27 different agents could be represented by three identifiers in base 3 code as:

	Agent 1	Agent 2	Agent 3	Agent 4
10 T1,T2,T3	0,0,0	1,0,0	2,0,0	0,1,0
	Agent 5	Agent 6	...	Agent 27
T1,T2,T3	1,1,0	2,1,0	...	2,2,2

To make such higher order encoding schemes practical, one additional identifier at a given quantity (e.g., ~1.0 pmol/bead) would be added to all members of the library to provide a standard against which the quantities of all identifiers would be measured. The quantities of the identifiers could be measured by gas chromatography or HPLC with a variety of detection methods. In the case of HPLC, quantities could be conveniently measured by scintillation counting if the identifiers were radioactively labeled by different quantities of a radionuclide such as tritium ( $^3\text{H}$ ). It would be particularly convenient to carry out the quantitation by measuring the  $^3\text{H}$ -to- $^{14}\text{C}$  ratio, thus using  $^{14}\text{C}$  as a standard. In this way, as many as ten quantities of  $^3\text{H}$  could be distinguished to create a base 10 or decimal code ( $M=10$ ) which could encode enormous amounts of information with very few identifiers.

30

-17-

Products and Synthetic Strategies

For the most part, the products of the method of this invention will be organic compounds where the serial synthesis will involve the addition or removal of chemical units, reactions involving the modification or introduction of one or more functionalities, ring openings, ring closings, etc. Chemical units can take many forms, both naturally-occurring and synthetic, such as nucleophiles, electrophiles, dienes, alkylating or acylating agents, diamines, nucleotides, amino acids, sugars, lipids, or derivatives thereof, organic monomers, synthons, and combinations thereof. Alternatively, reactions may be involved which result in alkylation, acylation, nitration, halogenation, oxidation, reduction, hydrolysis, substitution, elimination, addition, and the like. This process can produce non-oligomers, oligomers, or combinations thereof in extremely small amounts, where the reaction history, and composition in appropriate cases, can be defined by the present tags. Non-oligomers include a wide variety of organic molecules, e.g. heterocyclics, aromatics, alicyclics, aliphatics and combinations thereof, comprising steroids, antibiotics, enzyme inhibitors, ligands, hormones, drugs, alkaloids, opioids, terpenes, porphyrins, toxins, catalysts, as well as combinations thereof. Oligomers include oligopeptides, oligonucleotides, oligosaccharides, polylipids, polyesters, polyamides, polyurethanes, polyureas, polyethers, poly (phosphorus derivatives) e.g. phosphates, phosphonates, phosphoramides, phosphonamides, phosphites, phosphinamides, etc., poly (sulfur derivatives) e.g. sulfones, sulfonates, sulfites, sulfonamides, sulfenamides, etc., where for the phosphorous and sulfur derivatives the indicated heteroatom for the most part will be bonded to C, H, N, O or S, and combinations thereof.

-18-

Reactions may involve modifications at a variety of random sites of a central core molecular structure or modifications at a specific site. For example, one may brominate a polycyclic compound, where bromination may occur at a plurality of sites or use a brominating agent which will be specific for a particular site, e.g., N-bromosuccinimide. For the most part, reactions will involve single sites or equivalent sites, for example, one of two hydroxyl groups of a glycol.

For the most part, the subject synthesis will have at least two stages where other than bifunctional compounds are attached using the same linking functionality, e.g. amino acids and amide bonds, nucleotides and phosphate ester bonds, or mimetic compounds thereof, e.g., aminoisocyanates and urea bonds.

The methods of the invention permit variation in reaction at each stage, depending on the choice of agents and conditions involved. Thus, for amino acids, one may have up to 20 amino acids involved using the common naturally-encoded amino acids and a much wider choice, if one wishes to use other amino acids, such as D-amino acids, amino acids having the amino group at other than the  $\alpha$ -position, amino acids having different substituents on the side chain or substituents on the amino group, and the like. For the different nucleic acids, there will usually be up to 4 natural nucleic acids used for either DNA or RNA and a much larger number is available if one does not choose to use those particular nucleic acids. For the sugars and lipids, there are a very large number of different compounds, which compounds may be further increased by various substitutions, where all of these compounds may be used in the synthesis. For individual organic compounds the choice may be astronomically large. In addition, one may have mimetic analogs, where ureas, urethanes, carbonylmethylene groups, and the like may substitute for



-19-

the peptide linkage; various organic and inorganic groups may substitute for the phosphate linkage; and nitrogen or sulfur may substitute for oxygen in an ether linkage or vice versa.

5

The synthetic strategies will vary with the nature of the group of products one wishes to produce. Thus, the strategy must take into consideration the ability to stage-wise change the nature of the product, while  
10 allowing for retention of the results of the previous stages and anticipating needs for the future stages. Where the various units are of the same family, such as nucleotides, amino acids and sugars, the synthetic strategies are relatively well-established and frequently  
15 conventional chemistry will be available. Thus, for nucleotides, phosphoramidite or phosphite chemistries may be employed; for oligopeptides, Fmoc or Boc chemistries may be employed where conventional protective groups are used; for sugars, the strategies may be less conventional,  
20 but a large number of protective groups, reactive functionalities, and conditions have been established for the synthesis of polysaccharides. For other types of chemistries, one will look to the nature of the individual unit and either synthetic opportunities will be known or  
25 will be devised, as appropriate.

In some instances, one may wish to have the same or different blocks introduced at the same or different stages. For example, one may wish to have a common  
30 peptide functional unit, e.g. the fibronectin binding unit (RGDS), a polysaccharide, e.g. Le<sup>x</sup>, an organic group, e.g. a lactam, lactone, benzene ring, olefin, glycol, thioether, etc. introduced during the synthesis. In this manner one may achieve a molecular context into which the  
35 variation is introduced. These situations may involve only a few stages having the plurality of choices, where a large number of products are produced in relation to a

-20-

particular functional entity. This could have particular application where one is interested in a large number of derivatives related to a core molecule or unit known to have a characteristic of interest.

5

In developing synthetic strategies, one can provide for batch synthesis of a few compounds which would be prepared during the course of the combinatorial synthesis. By taking extreme examples, for example, syntheses which might involve steric hindrance, charge and/or dipole interactions, alternative reaction pathways, or the like, one can optimize conditions to provide for enhanced yields of compounds which might not otherwise be formed or be formed only in low yield. In this manner, one may allow for a variety of reaction conditions during the combinatorial synthesis, involving differences in solvent, temperatures, times, concentrations, and the like. Furthermore, one may use the batch syntheses, which will provide much higher concentrations of particular products than the combinatorial synthesis, to develop assays to characterize the activity of the compounds.

Supports: Attachment and Detachment

The synthetic protocol requires that one provide for a plurality of different reactions involving different reactants resulting in a plurality of different intermediates at each stage of the synthesis. While other techniques are available, this can be achieved most conveniently by employing small definable solid substrates, commercially available as beads, which can be readily mixed, separated, and serve as a solid substrate for the sequential synthesis. The solid substrates may be solid, porous, deformable or hard, and have any convenient structure and shape. In some instances, magnetic or fluorescent beads may be useful. The beads will generally be at least 10-2000  $\mu\text{m}$ , usually at least 20-500  $\mu\text{m}$ , more usually at least 50-250  $\mu\text{m}$  in diameter.

-21-

Any convenient composition can be used for the particles or beads, which bead composition will maintain its mechanical integrity during the various process stages, can be functionalized, has functional groups or allows for reaction with an active species, allows for the serial synthesis as well as attachment of the identifiers, can be readily mixed and separated, and will allow for convenient detachment of the tags and products. Beads which may be employed include cellulose beads, controlled-pore glass beads, silica gel, polystyrene beads, particularly polystyrene beads cross-linked with divinylbenzene, grafted co-polymer beads such as polyethyleneglycol/polystyrene, polyacrylamide beads, latex beads, dimethylacrylamide beads, particularly cross-linked with N,N'-bis-acryloyl ethylene diamine and comprising N-t-butoxycarbonyl- $\beta$ -alanyl-N'-acryloyl hexamethylene diamine, composites, such as glass particles coated with a hydrophobic polymer such as cross-linked polystyrene or a fluorinated ethylene polymer to which is grafted linear polystyrene; and the like. General reviews of useful solid supports (particles) that include a covalently-linked reactive functionality may be found in Atherton, et al., Prospectives in Peptide Chemistry, Karger, 101-117 (1981); Amamath, et al., *Chem. Rev.* 77:183-217 (1977); and Fridkin, The Peptides, Vol. 2, Chapter 3, Academic Press, Inc., (1979), pp. 333-363.

Depending upon the nature of the synthetic procedure or the assay of the final product, one or another bead may be more or less desirable. While beads are especially convenient, other solid supports may also find use, such as capillaries, hollow fibers, needles, solid fibers, etc., where the size of the solid support allows for the desired variation in reaction histories.

Depending upon the nature of the synthesis, the beads may be functionalized in a variety of ways to allow for

-22-

attachment of the initial reactant. These may be linked through a non-labile linkage such as an ester bond, amide bond, amine bond, ether bond, or through a sulfur, silicon, or carbon atom, depending upon whether one wishes to be able to remove the product from the bead. Conveniently, the bond to the bead may be permanent, but a linker between the bead and the product may be provided which is cleavable such as exemplified in Table 1. Two or more different linkages may be employed to allow for differential release of tags and/or products.

Depending upon the nature of the linking group bound to the particle, reactive functionalities on the bead may not be necessary where the manner of linking allows for insertion into single or double bonds, such as is available with carbenes and nitrenes or other highly-reactive species. In this case, the cleavable linkage will be provided in the linking group which joins the product or the tag to the bead.

Desirably, when the product is permanently attached, the link to the bead will be extended, so that the bead will not sterically interfere with the binding of the product during screening. Various links may be employed, particular hydrophilic links, such as polyethyleneoxy, saccharide, polyol, esters, amides, combinations thereof, and the like.

Functionalities present on the bead may include hydroxy, carboxy, iminoalide, amino, thio, active halogen (Cl or Br) or pseudohalogen (e.g.,  $-\text{CF}_3$ ,  $-\text{CN}$ , etc.), carbonyl, silyl, tosyl, mesylates, brosylates, triflates or the like. In selecting the functionality, some consideration should be given to the fact that the identifiers will usually also become bound to the bead. Consideration will include whether the same or a different functionality should be associated with the product and the identifier,

-23-

as well as whether the two functionalities will be compatible with the product or identifier attachment and tag detachment stages, as appropriate. Different linking groups may be employed for the product, so that a specific quantity of the product may be selectively released. In some instances the particle may have protected functionalities which may be partially or wholly deprotected prior to each stage, and in the latter case, reprotected. For example, amino may be protected with a carbobenzoxy group as in polypeptide synthesis, hydroxy with a benzyl ether, etc.

Where detachment of the product is desired, there are numerous functionalities and reactants which may be used. Conveniently, ethers may be used, where substituted benzyl ether or derivatives thereof, e.g. benzhydryl ether, indanyl ether, etc. may be cleaved by acidic or mild reductive conditions. Alternatively, one may employ  $\beta$ -elimination, where a mild base may serve to release the product. Acetals, including the thio analogs thereof, may be employed, where mild acid, particularly in the presence of a capturing carbonyl compound, may serve. By combining formaldehyde, HCl and an alcohol moiety, an  $\alpha$ -chloroether is formed. This may then be coupled with an hydroxy functionality on the bead to form the acetal. Various photolabile linkages may be employed, such as o-nitrobenzyl, 7-nitroindanyl, 2-nitrobenzhydryl ethers or esters, etc. Esters and amides may serve as linkers, where half-acid esters or amides are formed, particularly with cyclic anhydrides, followed by reaction with hydroxyl or amino functionalities on the bead, using a coupling agent such as a carbodiimide. Peptides may be used as linkers, where the sequence is subject to enzymatic hydrolysis, particularly where the enzyme recognizes a specific sequence. Carbonates and carbamates may be prepared using carbonic acid derivatives, e.g. phosgene, carbonyl diimidazole, etc. and a mild base. The link may

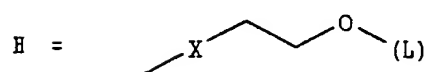
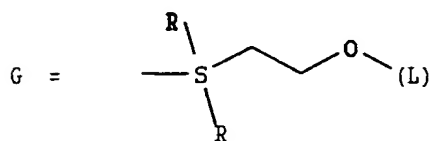
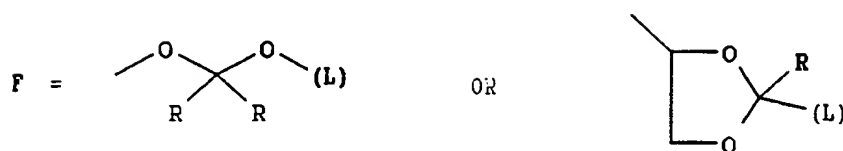
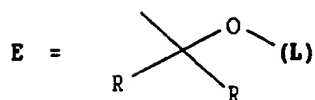
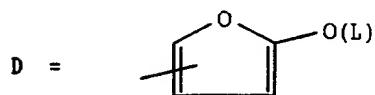
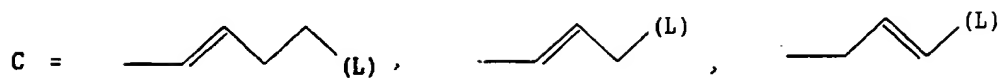
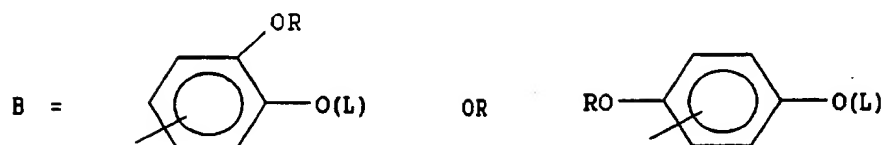
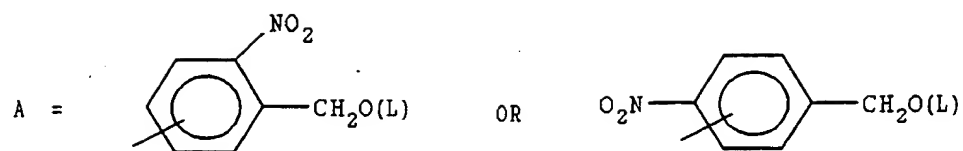
- be cleaved using acid, base or a strong reductant, e.g.,  $\text{LiAlH}_4$ , particularly for the carbonate esters. For a list of cleavable linkages, see, for example, Greene and Wuts, *Protective Groups in Organic Synthesis*, 2nd ed. Wiley, 1991. The versatility of the various systems that have been developed allows for broad variation in the conditions for attachment of products and identifiers and differential detachment of products and tags, as desired.
- 10 The following table indicates various illustrative linking units (i.e.,  $\text{F}^2$  in Formula I) and the manner in which they may be cleaved:

**Table 1.** Various illustrative linking units and the manner in which they may be cleaved.

	Linking Group	Cleavage Reagent
5	silyl	fluoride or acid
	A	$h\nu$
	B	$\text{Ce}(\text{NH}_4)_2(\text{NO}_3)_6$
	$-\text{NCO}_2(\text{L})^*$	$\text{OH}^-$ , $\text{H}^+$ , or $\text{LiAlH}_4$
10	C	$\text{O}_3$ , $\text{OsO}_4/\text{IO}_4^-$ , or $\text{KMnO}_4$
	D	1) $\text{O}_2$ or $\text{Br}_2$ , $\text{MeOH}$ 2) $\text{H}_3\text{O}^+$
	$-\text{Si}-(\text{L})$	oxidation, $\text{H}^+$ , $\text{Br}_2$ , $\text{Cl}_2$ , etc.
	E	$\text{H}_3\text{O}^+$
	F	$\text{H}_3\text{O}^+$
15	G	$\text{F}^-$ or $\text{H}^+$
	H	base, $\text{OH}^-$
20	x = keto, ester, amide, $\text{NO}_2$ , sulfide, sulfoxide, sulfone, and related electron withdrawing groups	
	I	$\text{H}_3\text{O}^+$ or reduction (e.g. $\text{Li}/\text{NH}_3$ )
	J	$(\phi_3\text{P})_3\text{RhCl}(\text{H})$
25	K	$\text{Li}$ , $\text{Mg}$ , or $\text{BuLi}$
	M	$\text{Hg}^{+2}$
	N	$\text{Zn}$ or $\text{Mg}$
30	x = halogen or pseudohalogen	
	O	oxidation (e.g. $\text{Pb}(\text{OAc})_4$ or $\text{H}_5\text{IO}_6$ )
	P	base
35	x = electron withdrawing group	

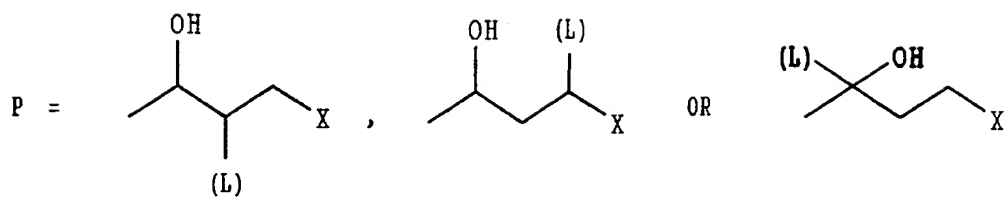
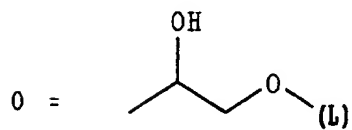
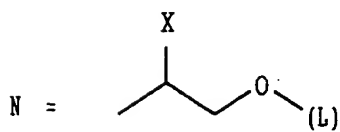
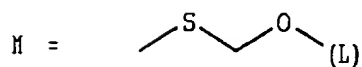
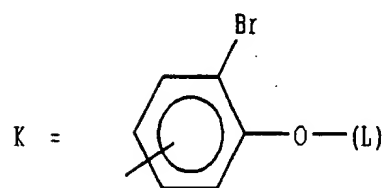
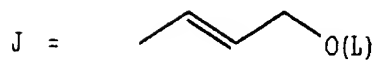
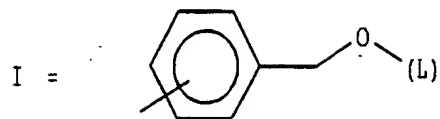
\* (L) shows the point of attachment of the tag or product.

- 26 -





-27-



-28-

(L) is the tag or product either directly bonded to the indicated atom or indirectly bonded through a linking group such as C(O)O, which linking group may provide a convenient functionality.

5 R is H or lower alkyl.

Linker.

The choice of linker for the ligand will be part of the synthetic strategy, since the linking group may result in a residual functionality on the product. It is feasible to further modify the product after detachment from the bead. In designing the synthetic strategy, one can use a functionality to be retained in the product as the point of attachment for the linking group. Alternatively, when permitted by the nature of the product, one could use a cleavage or detachment method which removes the linking functionality, e.g., an arylthioether or silyl with a metal hydride or acid. Since in many cases the synthetic strategy will be able to include a functionalized site for linking, the functionality can be taken advantage of in choosing the linking group. In some instances it may be desirable to have different functionalities at the site of linking the product to the support, which may necessitate using different modes of linking, which modes must accommodate either the same detachment method or different detachment methods which may be carried out concurrently or consecutively, e.g., irradiation with light and acid hydrolysis.

30 Of particular interest for binding the identifiers to the particle are carbenes and nitrenes which can insert between a carbon and hydrogen atom to form a covalent bond, or into an olefinic bond to form a cyclopropane (in the case of carbene) or an aziridine (in the case of nitrene).

35

-29-

With carbene or nitrene linking groups various substituted benzenes may be used, where the benzene is substituted with a group capable of providing a carbene:  $\text{CHN}_2$ ,  $\text{COCHN}_2$ ,  $\text{SO}_2\text{CHN}_2$ ; or nitrene:  $\text{N}_3$ ,  $\text{NO}_2$ ,  $\text{NO}$ ,  $\text{SO}_2\text{N}_3$ . The carbenes may be generated from diazoalkane derivatives by photolysis, thermolysis, or by treatment with low valent transition metal species, e.g.,  $\text{Rh}(\text{OAc})_2$ . The nitrene may be generated by photolysis or thermolysis from azides; and from nitro, nitroso and azides by using trivalent phosphorus compounds or low valent transition metals.

A group of linker moieties ( $\text{F}^1\text{-F}^2\text{-}$ ) of particular interest include 2-nitro-4-carboxybenzyloxy, 2-nitro-4-diazoacetylbenzyloxy, 4 or 5 azidomethylcarbonyl-2-methoxyphenoxy, and 2-methoxy-4, or 5-carboxyphenoxy moieties.

Illustrative compounds where T represents the tag, Z represents a carbene or nitrene precursor or a carboxy group, and R is H or lower alkyl are as follows. For photochemical tag detachment (e.g., with ultraviolet light at about 350 nm): T 3-Z-2-nitrobenzyl ether, T 4-Z-2-nitrobenzyl ether, T 5-Z-2-nitrobenzyl ether, T 6-Z-2-nitrobenzyl ether, T 2-Z-4-nitrobenzyl ether, T 3-Z-4-nitrobenzyl ether, T 3-Z-2-nitrobenzyl carbonate, T 4-Z-2-nitrobenzyl carbonate, T 5-Z-2-nitrobenzyl carbonate, T 6-Z-2-nitrobenzyl carbonate, T 2-Z-4-nitrobenzyl carbonate, and T 3-Z-4-nitrobenzyl carbonate. For oxidative detachment (e.g., using ceric ammonium nitrate): 1-OT-2-OR-3-Z-benzene, 1-OT-2-OR-4-Z-benzene, 1-OT-2-OR-5-Z-benzene, 1-OT-2-OR-6-Z-benzene, 1-OT-4-OR-2-Z-benzene, and 1-OT-4-OR-3-Z-benzene. For reductive or alkylative detachment (e.g. with lithium/ammonia or methyl iodide): T (2-Z-phenyl)thioether, T (3-Z-phenyl)thioether, and T (4-Z-phenyl)thioether. For desilylative detachment (e.g., using tetrabutyl ammonium fluoride or acid): T dialkyl-(2-Z-phenyl)silyl ether, T dialkyl-(3-Z-phenyl)silyl

-30-

ether, T dialkyl-(4-Z-phenyl)silyl ether, T-dialkyl-(2-Z-phenyl)silane, T-dialkyl-(3-Z-phenyl)silane, and T-dialkyl-(4-Z-phenyl)silane.

5     Combinatorial Synthesis

10     The synthesis will usually involve stages involving at least 2 choices, frequently at least 4 choices, and may involve 10 choices or more. Generally, the number of choices per stage will not exceed about 100, more usually not exceed about 50. The number of stages will usually be at least about 3, more usually at least about 4, frequently at least 5, and not more than about 30, more usually not more than about 25, preferably not more than about 20, more preferably not more than about 10, frequently not more than about 8.

20     The number of choices and stages will usually result in at least a number of compounds which allows for a sufficient variety to provide a reasonable likelihood that at least one compound will have the characteristic of interest. The subject methodology allows for producing greater than 25,000 compounds, usually greater than 50,000 compounds, preferably greater than 200,000 compounds, and a million or more may be produced. This will usually mean at least 25     20 compounds but may be  $10^6$  or more.

30     In some syntheses, a stage may only involve one or two choices, but this situation will usually be limited in relation to the number of compounds one wishes to produce and the particular synthetic strategy. In many of the strategies, the restricted number of choices, i.e., fewer than 5 choices, more usually 2 or fewer choices, will be limited to the greater of 40% of the total number of stages or about 2 stages in the sequential synthesis, more 35     usually limited to 20% of the total number of stages.

-31-

Reaction Procedure.

In carrying out the synthesis, one may initially begin with a number of beads, usually at least  $10^3$ , more usually  $10^4$ , and desirably at least  $10^5$ , while generally not exceeding  $10^{15}$ , more usually not exceeding at least  $10^{10}$ . Depending upon the number of choices in the first stage, one will divide up the particles accordingly into as many containers. One can use microtiter well plates, individual containers, columns, gels, Terasaki plates, flasks, Merrifield synthesis vessels, etc. The particles will usually be divided up into groups of at least one particle each, usually a plurality of particles, generally 1000 or more, and may be  $10^5$  or more depending on the total number of particles and choices involved in the stage.

One would then add the appropriate agents to each of the individual containers to process them in stages and add the identifiers which encode the reagent and stage. Each stage would provide the desired reaction. Once the reaction(s) is complete, one may wish to wash the beads free of any reagent, followed by combining all of the beads into a single mixture and then separating the beads according to the number of choices for the next stage. This procedure of dividing beads, followed by the tagging and synthesis stages (or vice versa), and then recombining beads is iterated until the combinatorial synthesis is completed.

In some instances, the same reaction may be carried out in 2 or more containers to enhance the proportion of product having a particular reaction at a particular stage as compared to the other choices. In other instances, one or more of the stages may involve a portion of the beads being set aside and undergoing no reaction, so as to enhance the variability associated with the final product. In other situations, batches may be taken along different synthetic pathways.

-32-

In order to record or encode the synthesis history on the beads, at each stage one would tag the beads associated with each choice and stage with their own unique combination of identifiers. Alternately one may use a single tag to record or encode this synthesis history. Depending on the chemistries involved, this tagging may be done prior to, after, or concomitantly with the reactions which comprise each choice. Further, as a control, sample beads may be picked at any stage and a portion of their tags cleaved off and decoded to verify that the correct tags are bound to the sample beads.

As indicated previously, in some instances, portions of the particles will be segregated into subsets, where each of the subsets would then undergo a different reaction series. At any time, the portions may be recombined into a single mixture for subsequent reaction. For example, if at one stage one introduces unsaturation, one could provide two subsets, where in one subset the unsaturation is reduced, while in the other subset the unsaturation is epoxidized. These two subsets could then be subjected to different reaction series.

After synthesis of the products is complete, they are screened for a desired property either after detachment of the ligand from the bead or while still attached. In the latter case, beads, for example, may be incubated in aqueous buffer with mouse monoclonal antibody Y. After incubation and washing, the beads are incubated with alkaline phosphatase-conjugated rabbit (or goat) polyclonal antibody directed against mouse antibodies. Using a fluorescent precipitation developing reagent, fluorescent beads with attached monoclonal antibody are identified and manually separated from the majority of clear, unstained beads. Alternatively, the fluorescent beads can be separated using a fluorescence-activated cell sorter, so long as the tags are retained on the bead under

-33-

the conditions of sorting. Each selected fluorescent bead is subjected to a means for releasing at least some portion of all of the tags from the bead.

5 In instances where the synthesis does not involve the stagewise addition of like units, or where reaction byproducts are formed, there may be instances where there will be a plurality of compounds on a single bead or the structure of the active compound cannot be known from its  
10 reaction history. In accordance with the subject invention, by knowing the reaction history, one may repeat the synthesis on a larger scale so as to obtain a sufficient amount of the product(s) to isolate the product(s) and structurally identify the active compound.

15 The subject methodology may be illustrated using various reaction sequences. For example, barbiturates may be prepared by combining an aldehyde or ketone with an acetate ester to prepare a crotonate under Claisen  
20 conditions to provide an unsubstituted to tetrasubstituted crotonate. The crotonate may then be combined with a second acetate under Michael conditions, whereby a glutarate may be obtained having up to 6 substituents. The glutarate may then be combined with ammonia or  
25 monosubstituted amine to provide the barbiturate. By varying the aldehydes and ketones, the acetates and the amines, a great variety of barbiturates may be obtained. Where functionalities are present on one or more of the substituents, such as amino, carboxy, hydroxy, thiol, and  
30 the like, these groups may be protected or modified as desired.

In another example described by Bunin and Ellman, *J. Am. Chem. Soc.*, 114, 10997 (1992), benzodiazepines are  
35 produced. One begins the synthesis with different amino protected substituted 2-aminobenzophenones bound to individual particles through, for example, a 4'-oxy group.

-34-

To each different group of particles in different vessels, after deprotection, are added a different Fmoc-protected  $\alpha$ -amino acid, either naturally occurring or synthetic, under conditions where a peptide bond is formed. After  
5 deprotection, internal cyclization is caused, followed by alkylation on nitrogen with an alkylating agent. In only three stages, a very large number of benzodiazepines are prepared and the libraries may be screened for tranquilizing or other activity.

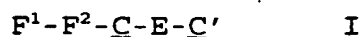
10

A wide variety of drug analogs may be produced, such as analogs of antihypertensive agents, e.g. enalapril;  $\beta$ -blockers, e.g. propranolol; antiulcer drugs ( $H_2$ -receptor antagonists) e.g. cimetidine and ranitidine; antifungal  
15 agents (cholesterol-demethylase inhibitors) e.g. isoconazole; anxiolytics, e.g. diazepam; analgesics, e.g. aspirin, phenacetamide, and fentanyl; antibiotics, e.g. vancomycin, penicillin and cephalosporin; antiinflammatories, e.g. cortisone; contraceptives, e.g.  
20 progestins; abortifacients, e.g. RU-456; antihistamines, e.g. chlorphenamine; antitussives, e.g. codeine; sedatives, e.g. barbitol; etc.

An illustrative synthesis of cimetidine analogs could  
25 involve hydroxymethylsubstituted histidines, and related heterocycles, where the remaining carbon atoms or nitrogen atoms could be further substituted or unsubstituted,  $\alpha,\omega$ -aminoalkylthiols, and substituted thioamide esters, where the groups on nitrogen could be varied, such as  
30 nitro, cyano, hydroxy, alkyl, combinations thereof, and the like.

#### Identifier

The identifiers of this invention may be represented by  
35 the Formula I:





-35-

where  $F^1$ - $F^2$  is a linker which allows for attachment to a support and detachment of the tag from a support; and

$\underline{C}$ - $E$ - $\underline{C}'$  is the tag which is capable of detection and distinguishability;

5         $E$  is a tag component which (a) allows for detection, such as an electrophoric group which can be analyzed by gas chromatography or mass spectroscopy or (b) allows for detection and for separation;

10         $\underline{C}$  and  $\underline{C}'$  are tag components which allow for individually distinguishing one tag from all other tags, usually allowing for separation as a result of variable length or substitution, for example, varying the chromatographic retention time or the mass spectroscopy ratio  $m/e$ ;

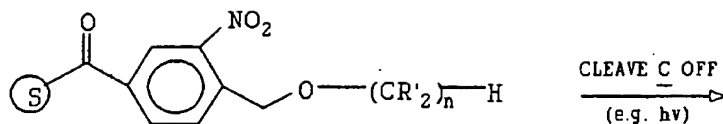
15         $F^2$  is a linking component capable of being selectively cleaved to release the tag component; and

$F^1$  is a functional group which provided for attachment to the support; or

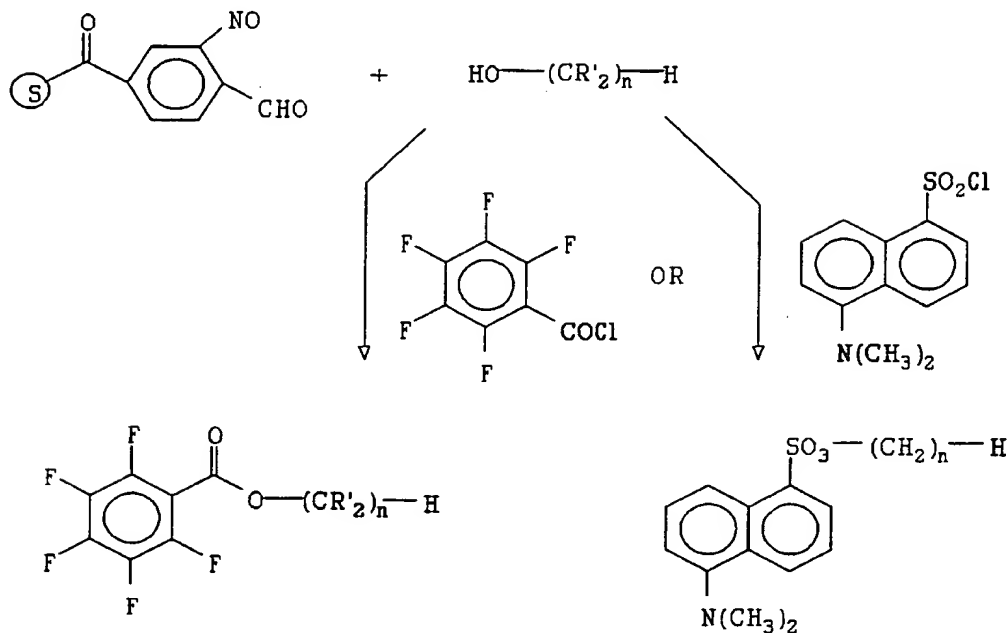
20         $F^2$  is a bond when  $F^1$  is a cleavable group such as OH or carboxy.

Although the identifiers of Formula I are typically added at each appropriate stage and choice during the combinatorial synthesis, the portion  $E$  can be added at the  
25        end of the syntheses either before or after cleavage (preferably photochemically or oxidatively) from the substrate. Specifically, where  $\underline{C}$  contains OH,  $NHR^4$ , or SH,  $E$  can be attached to  $\underline{C}$  prior to cleavage. Alternatively, if  $E$  is attached after cleavage, the point of attachment  
30        at  $\underline{C}$  may be where  $F^2$  was attached. This is exemplified in the scheme on the following page:

-36-



5



10

15

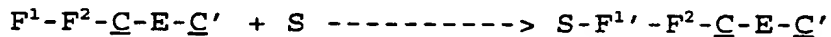
20

where S = substrate and

n = 1-40

Attachment of the identifier to the substrate can be represented as follows:

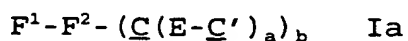
25



where  $F^1'-F^2-\underline{C}-E-\underline{C}'$  represents the identifier residue attached to the substrate. For example, when the bead is functionalized with an aminomethyl group and  $F^1$  is  $\text{CO}_2\text{H}$ , then  $F^1'$  is  $-\text{C}(\text{O})-$ ; when the bead contains an unsaturated bond and  $F^1$  is  $\text{N}_2\text{CH}-\text{C}(\text{O})-$ , then  $F^1'$  is  $=\text{CH}-\text{C}(\text{O})-$  or  $-\text{CH}_2-\text{C}(\text{O})-$ .

30

Of particular interest for use as identifiers are compounds of Formula I of the Formula Ia:



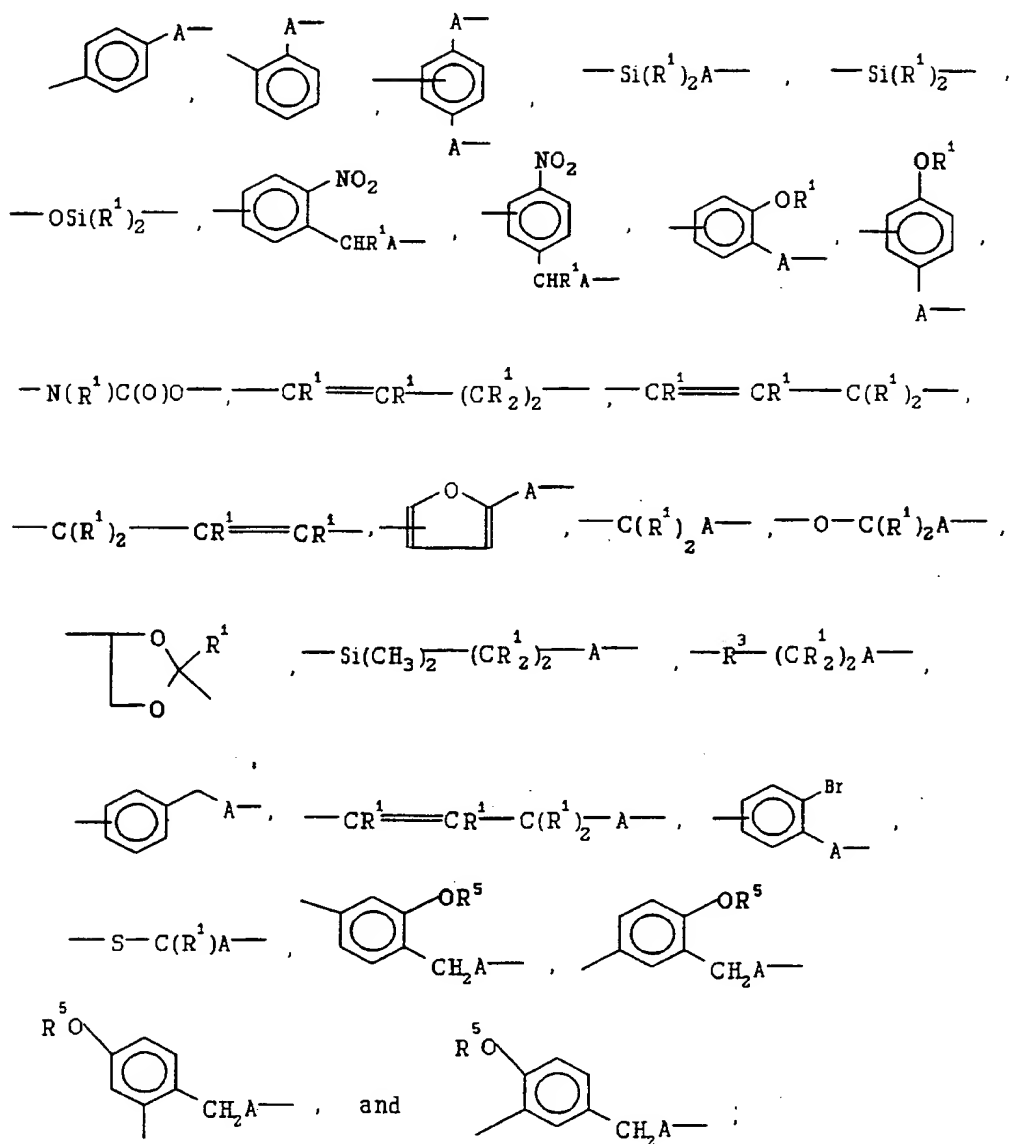
35

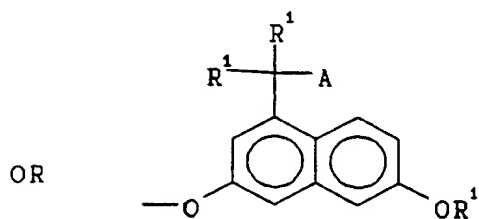
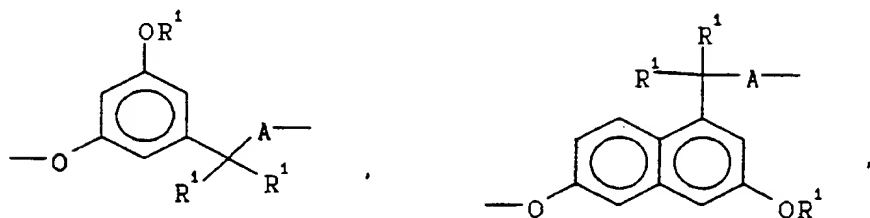
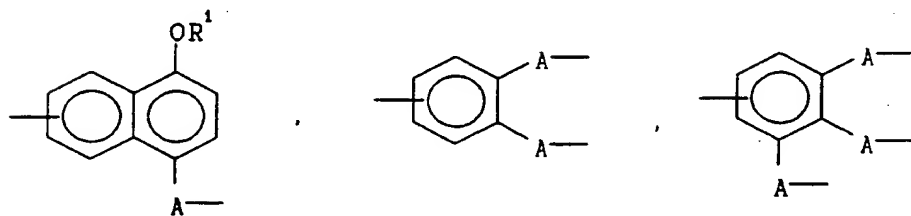
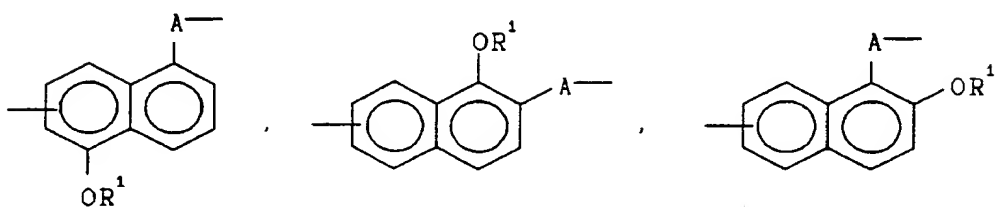
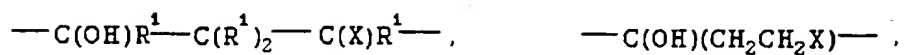
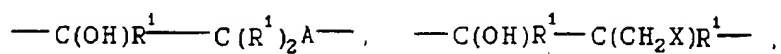
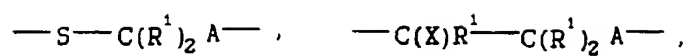
- 37 -

wherein:

$F^1$  is  $\text{CO}_2\text{H}$ ,  $\text{CH}_2\text{X}$ ,  $\text{NR}^1\text{R}^1$ ,  $\text{C}(\text{O})\text{R}^1$ ,  $\text{OH}$ ,  $\text{CHN}_2$ ,  $\text{SH}$ ,  $\text{C}(\text{O})\text{CHN}_2$ ,  
 $\text{S}(\text{O})_2\text{Cl}$ ,  $\text{S}(\text{O})_2\text{CHN}_2$ ,  $\text{N}_3$ ,  $\text{NO}_2$ ,  $\text{NO}$ ,  $\text{S}(\text{O})_2\text{N}_3$ ,  $\text{OC}(\text{O})\text{X}$ ,  $\text{C}(\text{O})\text{X}$ ,  
 $\text{NCO}$ , or  $\text{NCS}$ ;

5  $F^2$  is





-39-

- A is -O-, -OC(O)O-, -OC(O)-, or -NHC(O)-;
- C is a bond; C<sub>1</sub>-C<sub>20</sub> alkylene optionally substituted by 1-40 F, Cl, Br, C<sub>1</sub>-C<sub>6</sub> alkoxy, NR<sup>4</sup>R<sup>4</sup>, OR<sup>4</sup>, or NHR<sup>4</sup>, or -[(C(R<sup>4</sup>)<sub>2</sub>)<sub>m</sub>-Y-Z-Y-(C(R<sup>4</sup>)<sub>2</sub>)<sub>n</sub>Y-Z-Y]<sub>p</sub>-; with the proviso
- 5 that the maximum number of carbon atoms in C+C' is preferably 20;
- C' is H; F; Cl; C<sub>1</sub>-C<sub>20</sub> alkyl optionally substituted by 1-40 F, Cl, Br, C<sub>1</sub>-C<sub>6</sub> alkoxy, NR<sup>4</sup>R<sup>4</sup>, OR<sup>4</sup>, or NHR<sup>4</sup>, or -[(C(R<sup>4</sup>)<sub>2</sub>)<sub>m</sub>-Y-Z-Y-(C(R<sup>4</sup>)<sub>2</sub>)<sub>n</sub>Y-Z-Y]<sub>p</sub>-;
- 10 E is C<sub>1</sub>-C<sub>10</sub> alkyl substituted by 1-20 F, Cl or Br; or Q-aryl wherein the aryl is substituted by 1-7 F, Cl, NO<sub>2</sub>, SO<sub>2</sub>R<sup>5</sup>, or substituted phenyl wherein the substituent is 1-5 F, Cl, NO<sub>2</sub>, or SO<sub>2</sub>R<sup>5</sup>;
- E-C' may be -H, -OH, or amino;
- 15 R<sup>1</sup> is H or C<sub>1</sub>-C<sub>6</sub> alkyl;
- R<sup>3</sup> is C=O, C(O)O, C(O)NR<sup>1</sup>, S, SO, or SO<sub>2</sub>;
- R<sup>4</sup> is H or C<sub>1</sub>-C<sub>6</sub> alkyl;
- R<sup>5</sup> is C<sub>1</sub>-C<sub>6</sub> alkyl;
- a is 1-5;
- 20 b is 1-3;
- m and n is each 0-20;
- p is 1-7;
- Q is a bond, O, S, NHR<sup>4</sup>, C=O, -C(O)NR<sup>5</sup>, -NR<sup>5</sup>C(O)-, -C(O)O-, or -OC(O)-;
- 25 X is a leaving group such as Br, Cl, triflate, mesylate, tosylate, or OC(O)OR<sup>5</sup>;
- Y is a bond, O, S, or NHR<sup>4</sup>;
- Z is a bond; phenylene optionally substituted by 1-4 F, Cl, Br, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> alkyl substituted
- 30 by 1-13 F, Cl, or C<sub>1</sub>-C<sub>6</sub> alkyloxy substituted by 1-13 F, Cl, or Br; (C(R<sup>4</sup>)<sub>2</sub>)<sub>1-20</sub>; or (CF<sub>2</sub>)<sub>1-20</sub>; with the proviso that when Z is a bond one of its adjacent Y's is also a bond; and
- aryl is a mono- or bi-cyclic aromatic ring containing up
- 35 to 10 carbon atoms and up to 2 heteroatoms selected from O, S, and N.

-40-

In the definitions of  $F^2$  in Formula Ia, the left-hand bond as depicted attaches to  $F^1$ .

Also useful as identifiers are compounds of the Formula Ia';



wherein:

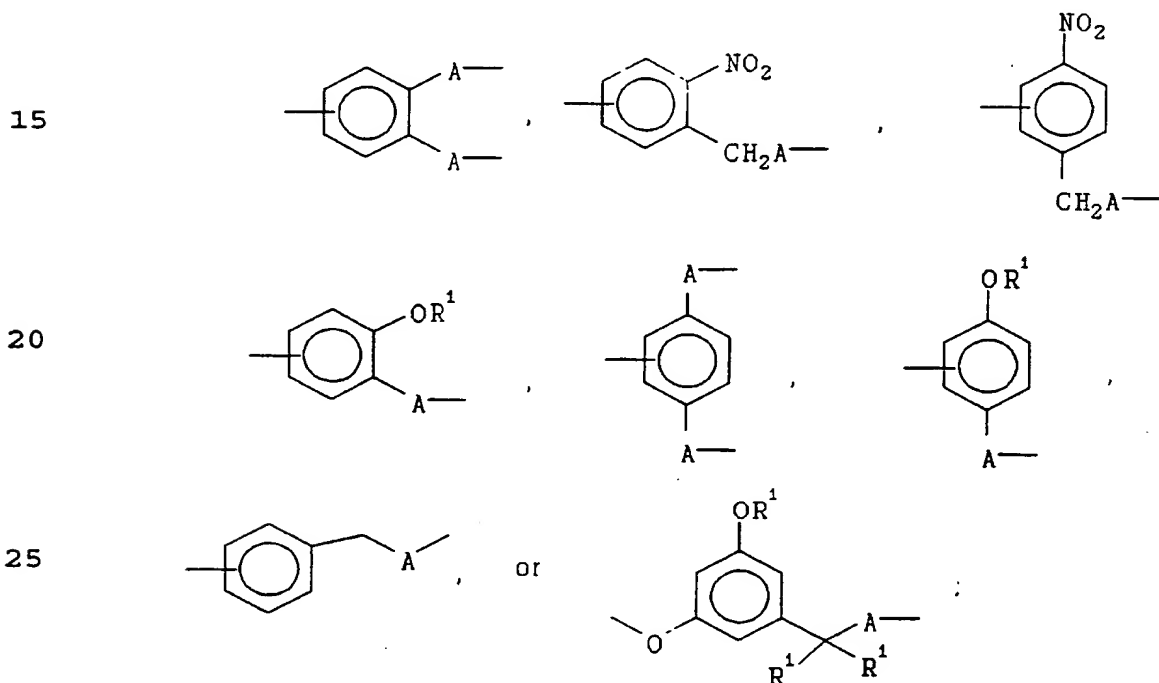
$F^1$  is OH or COOH; and

the remaining definitions are as in Formula Ia.

Preferred compounds of Formula Ia are those wherein;

10  $F^1$  is

$F^2$  is  $CO_2H$ , OH,  $CHN_2$ ,  $C(O)CHN_2$ ,  $C(O)X$ , NCS, or  $CH_2X$ :



$\underline{C}$  and  $\underline{C}'$  is each independently  $C_1$ - $C_{20}$  alkylene or  $C_1$ - $C_{20}$  alkyl, respectively, unsubstituted or substituted by 1-40 F or Cl, or  $[O-(CH_2)_{2-3}]_p$ ;

35 E is  $C_1$ - $C_{10}$  alkyl substituted by 1-20 F or Cl; Q-aryl where aryl is a bi-cyclic aromatic ring substituted by 1-7

-41-

F or Cl; or Q-phenyl substituted by 1-5 F, Cl, NO<sub>2</sub>, or SO<sub>2</sub>R<sup>5</sup>; and

Q is a bond, O, -NR<sup>5</sup>C(O)-, or -OC(O)-.

5 Preferred compounds of Formula Ia are those wherein

-C(E-C')<sub>a</sub> is represented by -(CH<sub>2</sub>)<sub>0-15</sub>-(CF<sub>2</sub>)<sub>1-15</sub>F,

-(CH<sub>2</sub>)<sub>0-15</sub>-(CCl<sub>2</sub>)<sub>1-15</sub>Cl, -(CH<sub>2</sub>CH<sub>2</sub>-O)<sub>1-5</sub>-Ar,

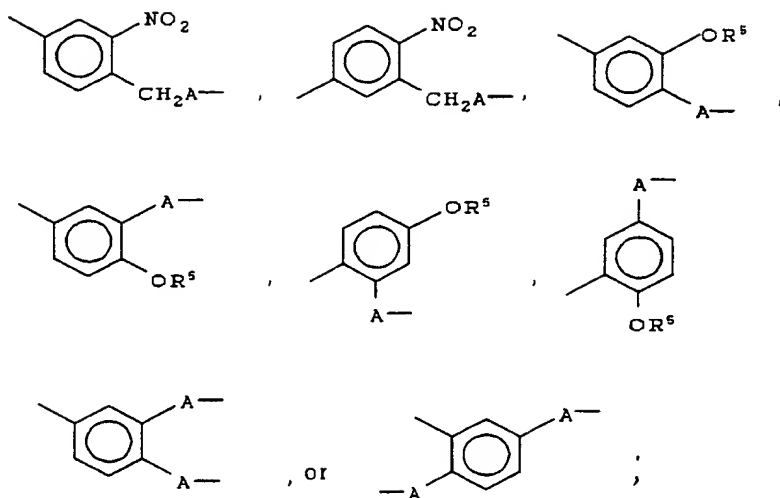
-(CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O)<sub>1-5</sub>-Ar, or -(CH<sub>2</sub>)<sub>1-12</sub>-O-Ar;

10 wherein Ar is pentafluoro- pentachloro-, or pentabromophenyl, 2,3,5,6-tetrafluoro-4(2,3,4,5,6-pentafluorophenyl)phenyl, 2,4,6-trichlorophenyl, 2,4,5-trichlorophenyl, 2,6-dichloro-4-fluorophenyl, or 2,3,5,6-tetrafluorophenyl.

15

A preferred embodiment of Formula Ia is wherein F<sup>1</sup> is COOH, CHN<sub>2</sub>, C(O)CHN<sub>2</sub>, S(O<sub>2</sub>)CHN<sub>2</sub>, COCl, OH, SH, CH<sub>2</sub>X, or NHR<sup>1</sup>;

-42-

wherein F<sup>2</sup> is

wherein A is -O- or -OC(O)O-;

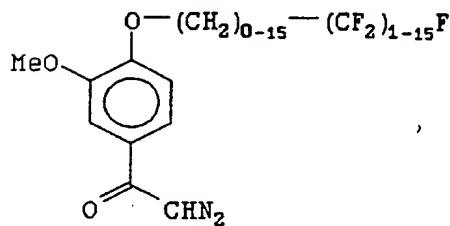
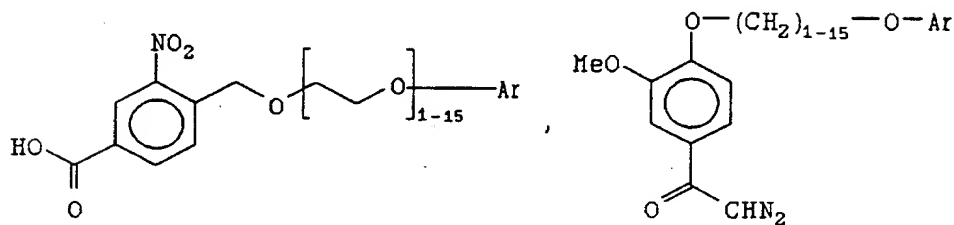
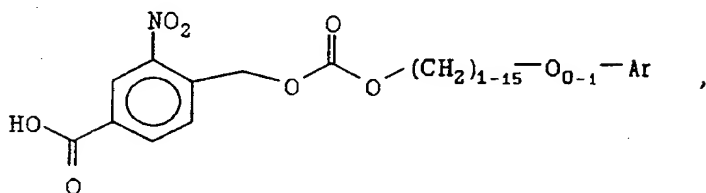
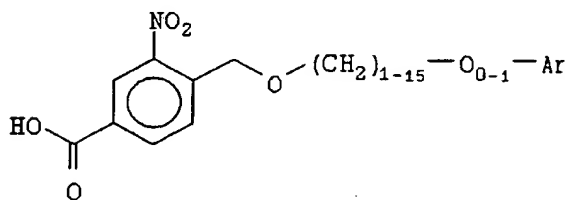
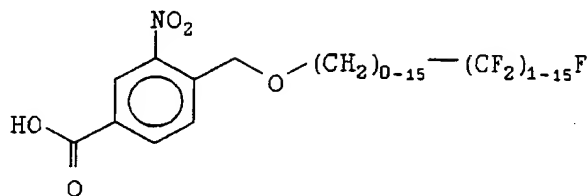
- 5      $\underline{C}(\underline{E}-\underline{C}')_a$  is  $-(\text{CR}^4_2)_{1-15}-(\text{O})_{0-1}-\text{Ar}$ ,  $-(\text{CR}^4_2)_{0-15}-(\text{CF}_2)_{1-15}-\text{F}$ ,  
        $-(\text{CR}^4_2)_{0-15}-(\text{CF}_2)_{1-15}-(\text{CR}^4_2)_{0-15}-\text{H}$ , or  
        $-(\text{CH}_2)_{1-20}-(\text{O})_{0-1}-(\text{CH}_2)_{1-19})_{0-24}-(\text{CH}_2)_{0-24}-(\text{O})_{0-1}-\text{Ar}$ ,

10     wherein Ar is pentafluoro-, pentachloro-, or  
       pentabromophenyl, 2,3,5,6- tetrafluoro-4(2,3,4,5,6-  
       pentafluorophenyl)phenyl, 2,4,6-trichlorophenyl,  
       2,4,5-trichlorophenyl, 2,6-dichloro-4-fluorophenyl,  
       or 2,3,5,6-tetrafluorophenyl.

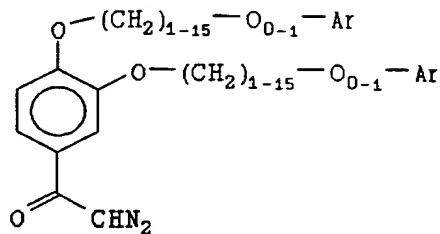


Other preferred compounds of Formula Ia are represented by the formulae:

5

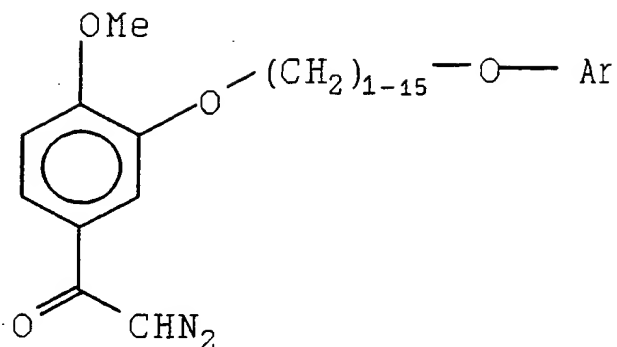


OR



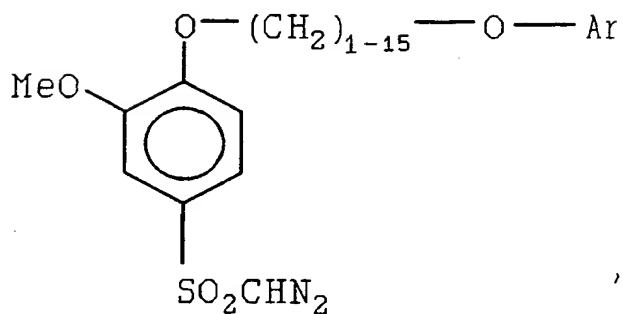
-44-

5



10

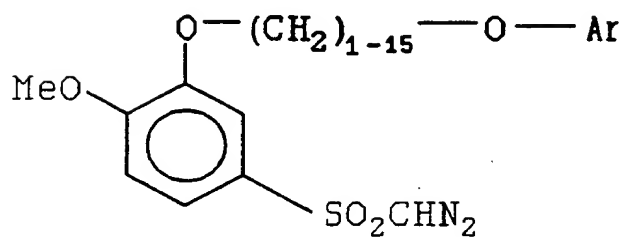
15



20

, and

25



wherein Ar is pentafluoro-, pentachloro-, or  
 30 pentabromophenyl, 2,3,5,6-tetrafluoro-4(2,3,4,5,6-  
 pentafluorophenyl)phenyl, 2,4,6-trichlorophenyl,  
 2,4,5-trichlorophenyl, 2,6-dichloro-4-fluorophenyl,  
 or 2,3,5,6-tetrafluorophenyl.

Other preferred compounds of Formula Ia are those

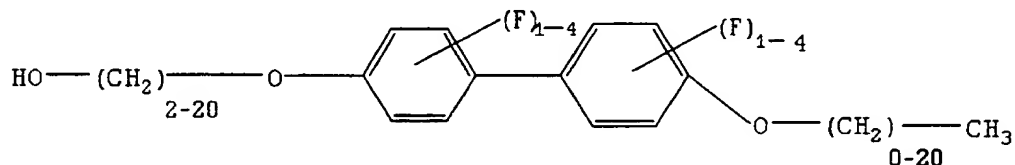
35

wherein E-C' is H, OH, or NH<sub>2</sub>. Such compounds are  
 particularly useful for reaction with an E at the end of

-45-

the combinatorial synthesis, especially with an E detectable by fluorescence or electron capture, such as dansyl chloride or polyhalobenzoylhalide.

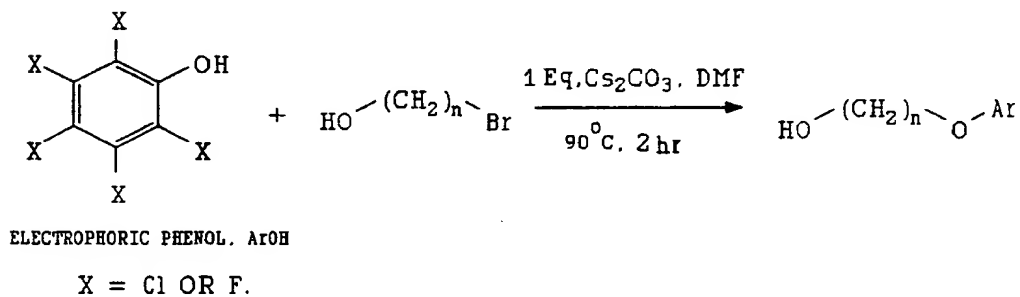
- 5 Another embodiment of the invention is represented by the following compound:



- 10 The compounds of Formula I can be prepared according to the following exemplary schemes or other means known to those skilled in the art.

### SCHEME 1

#### Identifier Tag Preparation



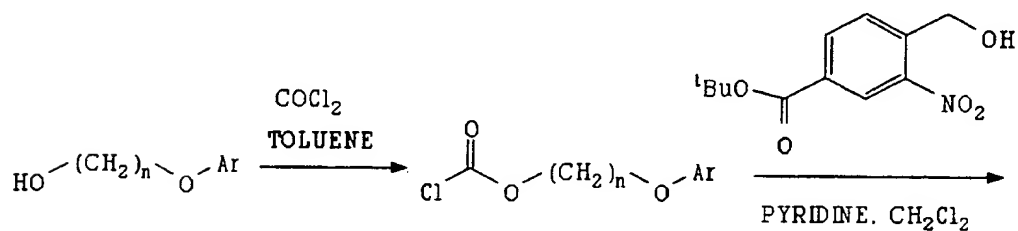
15

## SCHEME 2

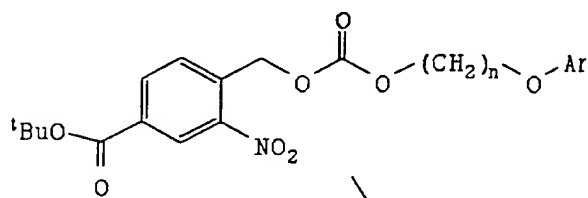
Identifiers With Photolytic Cleavage Linkers

5

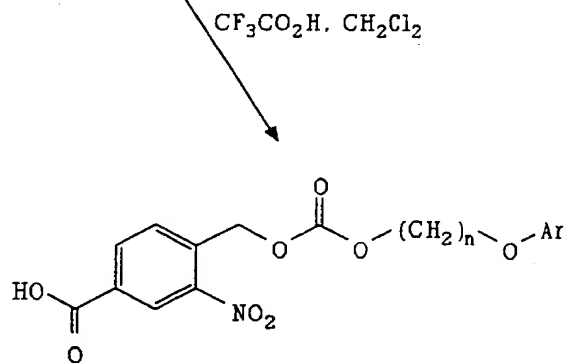
10



15



20

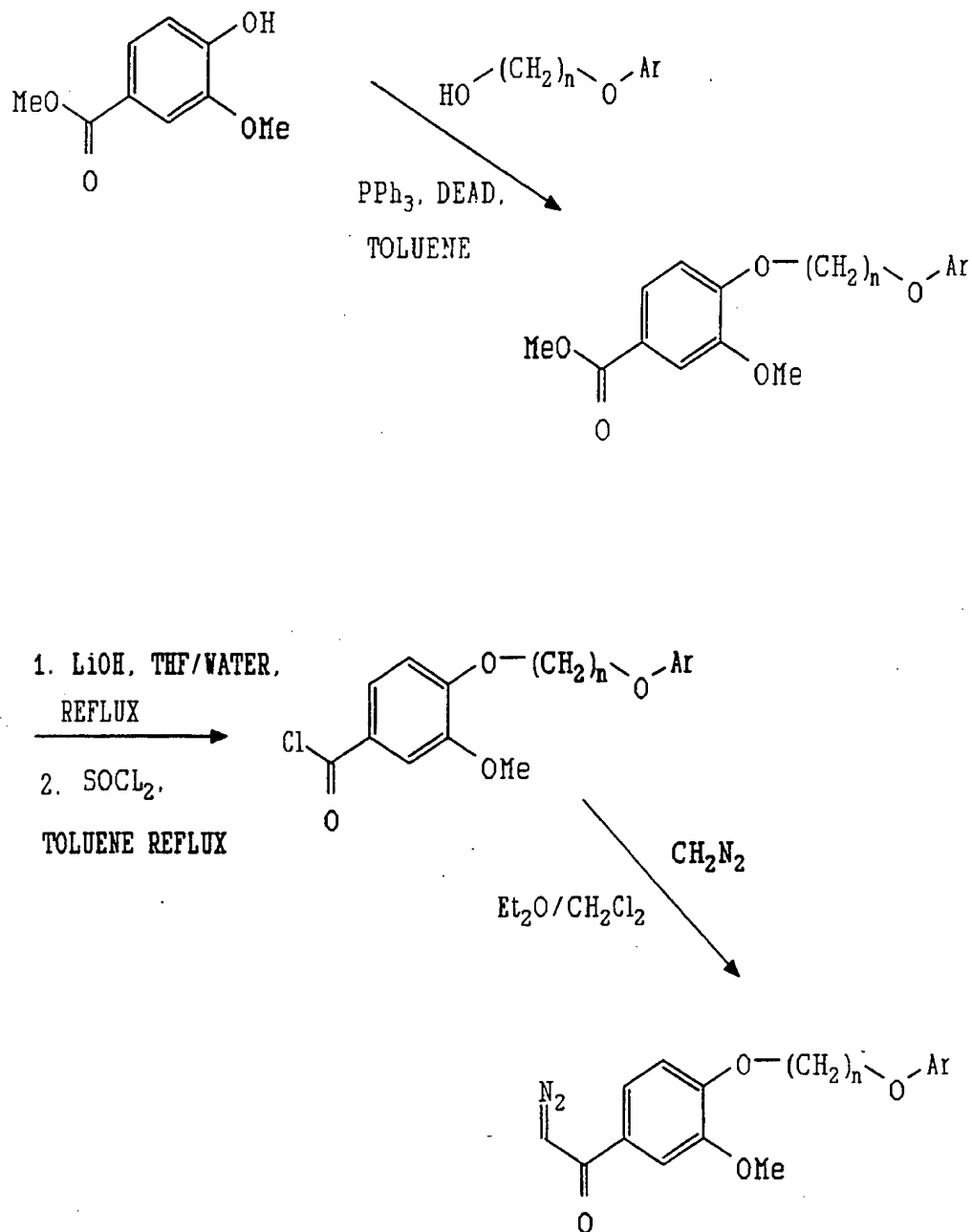


- 47 -

## SCHEME 3

Identifiers With Oxidative Cleavage Linkers

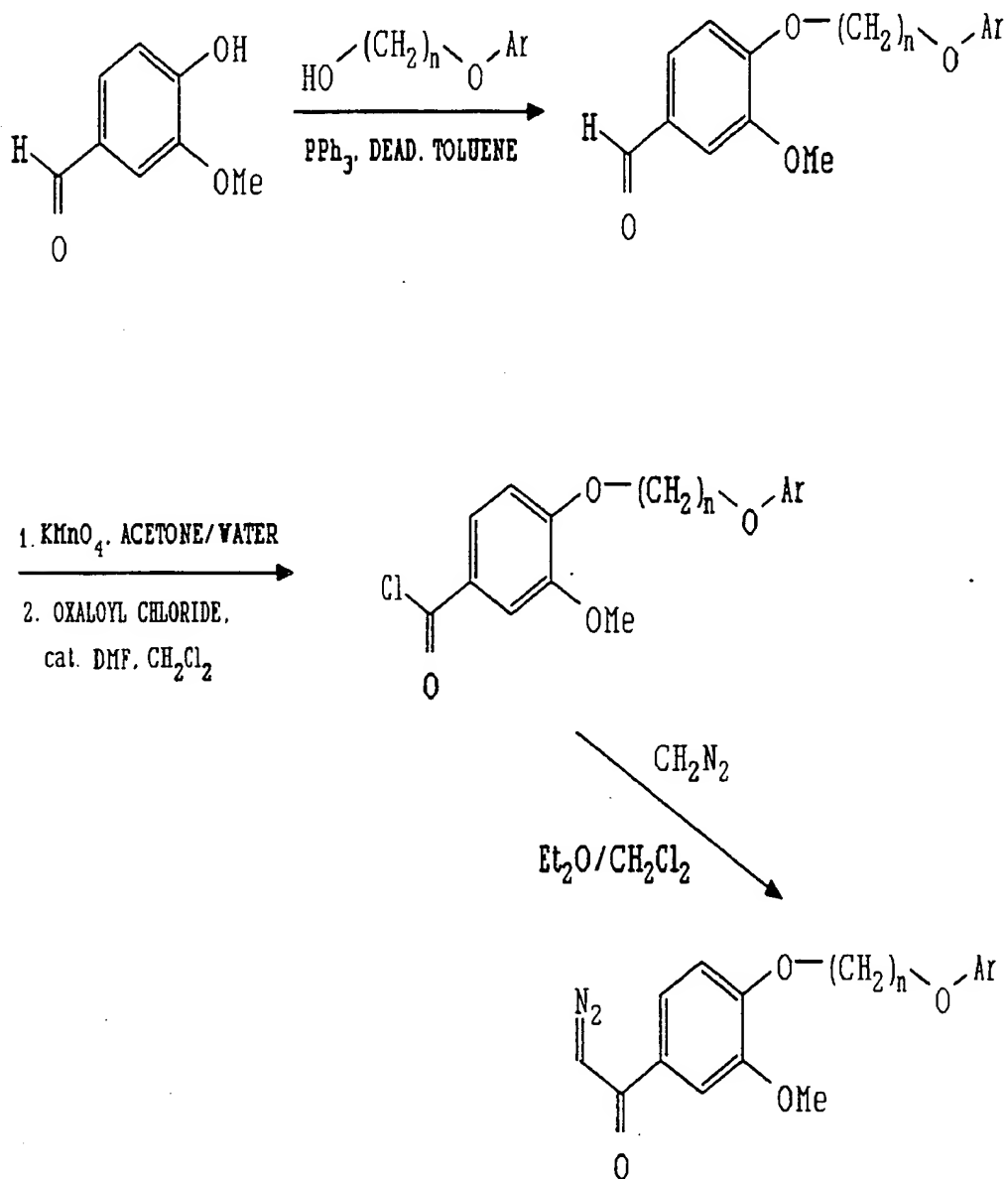
5



**SCHEME 4**

## Identifiers With Alternative Oxidative Release Linkers

5



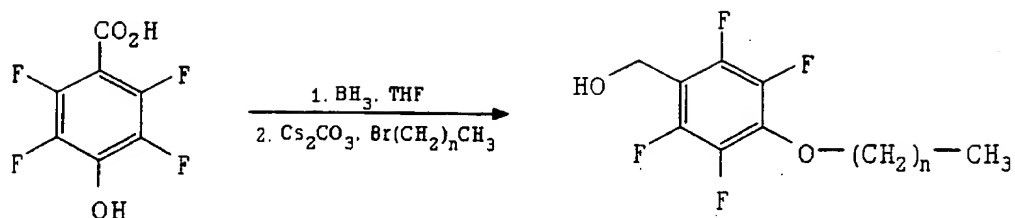
-49-

## SCHEME 5

E-C' Tags

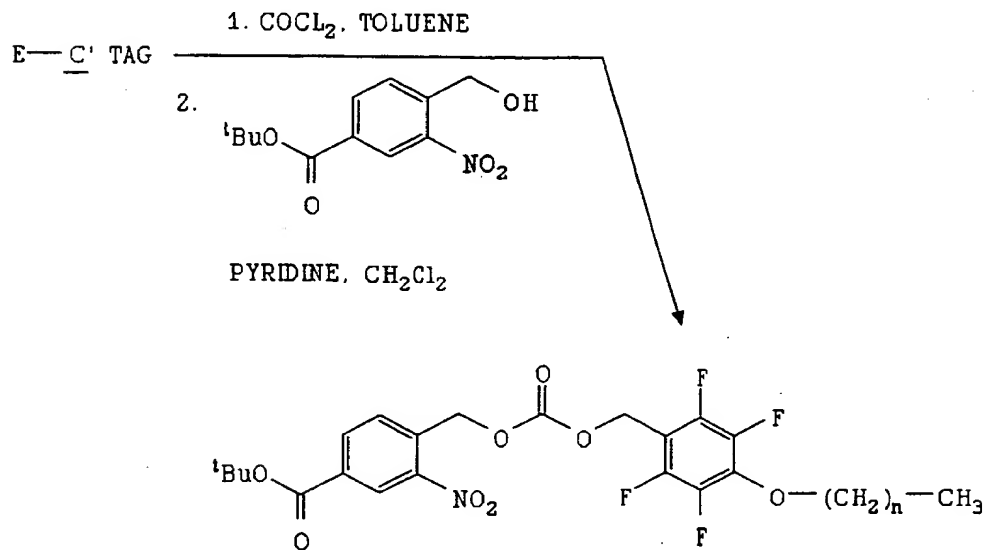
5

10



## SCHEME 6

15

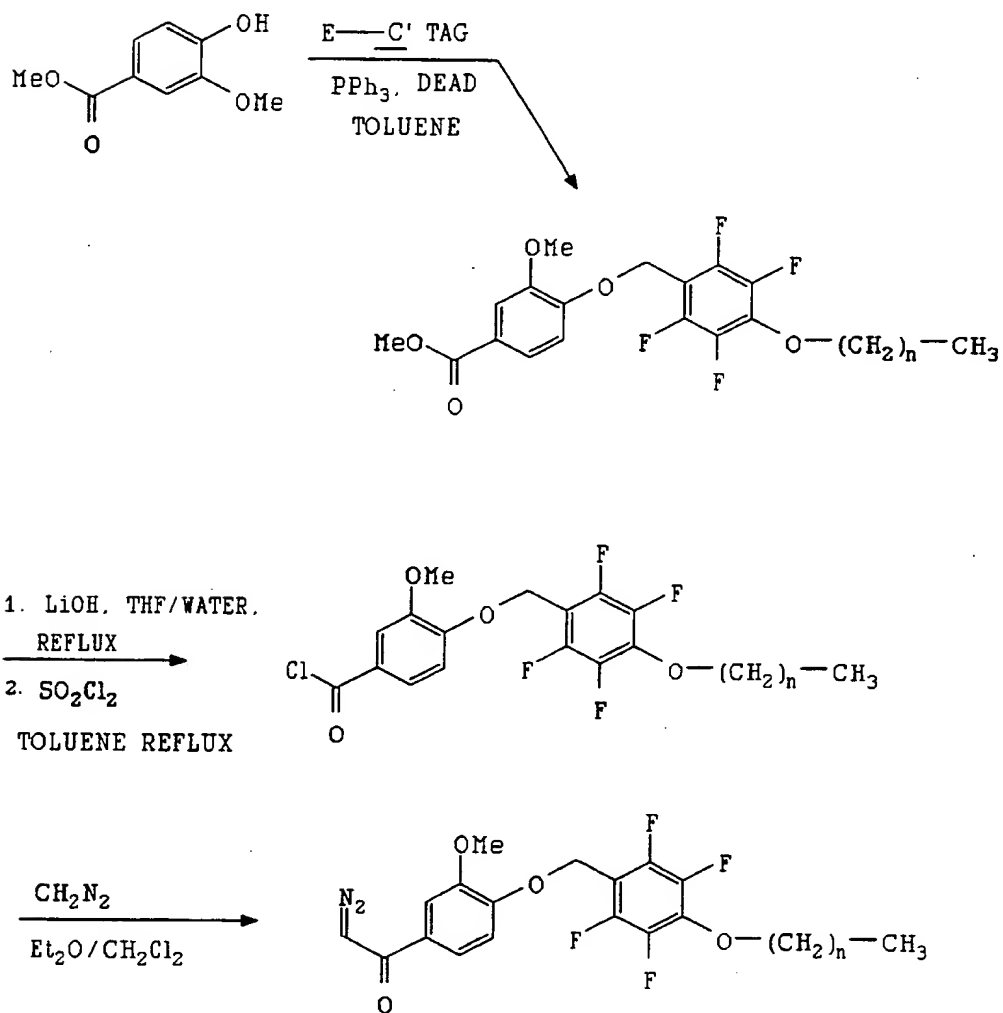
Identifiers With Photolytic Cleavage Linkers

-50-

## SCHEME 7

Identifiers With Oxidative Cleavage Linkers

5





-51-

5 The identifier may comprise one or a plurality of identical tags. The identifiers will be individual chemical compound(s) which may be distinguished one from the other and will uniquely identify different choices and stages. In this manner, very large combinatorial libraries may be prepared with a relatively small number of identifiers, usually fewer than 50 tags.

10 During each stage, a combination of identifiers will be added, which defines the stage and choice. Each identifier will be bound, either covalently or non-covalently to the bead or to the product, usually the bead. Combinations of identifiers are used to provide a binary or other code at each stage, whereby the choice and stage may be defined. The combination of identifiers may  
15 include zero or only one identifier.

#### Tags

20 So far as the tags (C-E-C') are concerned, the tags which are employed will be characterized as follows: by being removable from the bead by means depending on  $F^1$  or  $F^2$ , preferably by hydrolysis, photolysis or oxidation; by being individually differentiable, usually separable; by being stable under the synthetic conditions; by encoding  
25 both stage and choice so as to uniquely define the choice of agent used at each stage in the synthesis; desirably, there should be an easy way to identify the various tags with readily-available equipment which does not require sophisticated technical capabilities to operate; they  
30 should be relatively economical and provide a strong signal based on a relatively few molecules; and the tags should provide sufficient sensitivity to permit distinguishing the tags from the other components which may be present during the tag determinations.

35

The tags may be structurally related or unrelated, as in a homologous series, repetitive functional groups, related

-52-

members of the Periodic Chart, different isotopes, combinations thereof, or the like. The tags may be used as elements of a binary code, so that one tag can define two choices, two tags can define four choices, three tags  
5 can define eight choices, five tags can define thirty-two choices, etc. Thus, at each stage of the synthesis, a relatively small number of tags can designate a much larger number of choices. The tags comprising the identifiers for each stage may or may not be related to  
10 other stages. Each tag for any combinatorial synthesis must allow for being distinguished from all other tags. In this manner, very large combinatorial libraries may be prepared with a relatively small number of tags, usually fewer than 60 tags, more usually fewer than about 50 tags.

15 For each bead, there will usually be at least 0.01 femtomol, more usually 0.001-50 pmol, of each tag, although lesser or greater amounts may be used in special circumstances. The amount of product may also be at least  
20 in the same range and up to at least  $10^4$  or more greater, usually being at least 0.01 pmol, more usually at least 1.0 pmol and generally not more than about 10 nmol. Depending upon the number of beads, the number of stages and the number of choices per stage, the number of  
25 products produced will usually exceed  $10^2$ , more usually  $10^3$ , and may exceed  $10^{10}$ , usually not exceeding about  $10^6$ , preferably being in the range of about  $10^4$  to  $10^8$ , more usually  $10^5$  to  $10^8$ .

30 The tags will, for the most part, be organic molecules. Each tag will usually have fewer than about 100 atoms, more usually fewer than about 80 atoms, generally fewer than about 60 atoms, other than hydrogen, excluding a linking moiety which would not be retained on release of  
35 the tag from the bead. The linking moiety may be of any size, usually being fewer than about 30 atoms, more usually fewer than 20 atoms, other than hydrogen. The

-53-

size of the linking moiety is not critical, but one of convenience. The tags may form families of compounds, where all of the compounds are of a similar nature or may be combinations of different families, where the compounds  
5 may be aliphatic, alicyclic, aromatic, heterocyclic, or combinations thereof. Distinguishing features may be the number of repetitive units, such as methylene groups in an alkyl moiety, alkyleneoxy groups in a polyalkyleneoxy moiety, halo groups in a polyhalocompound,  $\alpha$ - and/or  $\beta$ -  
10 substituted ethylenes, where the substituents may involve alkyl groups, oxy, carboxy, amino, halo, or the like; isotopes; etc.

#### Tag Analysis

15 Tags may be removed from the bead using reductive, oxidative, thermolytic, hydrolytic, or photolytic conditions depending on the nature of the group F<sup>2</sup>; for example, by oxidation of a catechol ether with ceric ammonium nitrate or by photolysis of a nitrobenzyl ether  
20 or ester or amide, or by other methods, e.g. as shown in Table 1.

Differentiation of tags can be achieved with physical differences, e.g. molecular weight of the tags or the  
25 chromatographic retention time using gas or liquid chromatography. Positional isomers may have different retention times. If positional isomers or stereoisomers are inadequate for physical separation, then one could use varying numbers of substituents, e.g. halogens (such as  
30 fluorines), methyl groups, oxy groups, or other side chains in conjunction with differing numbers of units, e.g. methylene groups or ethyleneoxy groups, to provide the desired separation. Ratios of radioisotopes could be used, where the radioisotopes provide for differential  
35 emission, for example <sup>14</sup>C and <sup>3</sup>H. The physical differences, particularly mass number, can provide information about choice and stage.

-54-

Instead of  $^{14}\text{C}/^3\text{H}$  ratios, one could use combinations of non-radioactive isotopes, e.g.  $-\text{CH}_m\text{D}_n$ , where m is 0 and up to 3 and n is 3 minus m. For example, by detecting the varying amounts of up to four different methyl groups  
5 using mass spectroscopy, one could define a large number of choices.

When E-C' is H, the tags obtained upon release from the support have an active functionality for reaction with a  
10 labeling reagent which introduces a detectable tag component E. Conveniently, the functionality could be a double bond (particularly an activated double bond), hydroxy, thio, amino, carboxy, etc. The tag would then be reacted with an excess of the labeling reagent to provide  
15 the product (E-CH) for analysis. In this way a wide variety of labeling reagents could be used as part of the identifying system, which may not be compatible with the synthetic strategy for the product of interest. Labeling reagents which may be used for detection include  
20 haloaromatics (e.g., perfluorobenzyl bromide), fluorescers (e.g., dansyl chloride), radioisotopes, chemiluminescers, etc.

While exemplary tags and reactions have been given, it  
25 should be understood that many other combinations could be employed.

Depending on the chemical and physical nature of the tags, an appropriate method for separation is chosen, desirably  
30 one of various chromatographic procedures including gas chromatography (GC), liquid chromatography (LC) particularly high-performance liquid chromatography (HPLC), thin layer chromatography (TLC), electrophoresis, etc. Instead of a chromatographic procedure, mass  
35 spectrometry may be employed for separation by mass number. Tags include:

-55-

for GC: chemically inert organic molecules having the same or different molecular weights including alkanes, alkenes, arenes, halocarbons, ethers, alcohols, silanes, thioethers, etc., particularly halogenated compounds, with or without other functionalities, for electron capture detection or mass spectroscopy detection (MS) with capillary GC separation, and for compound with elements not normally found in organic chemistry (e.g., Sn, Ge) for atom emission detection with GC capillary separation;

for LC, HPLC or TLC: see above for GC, conveniently linear ethers or hydrocarbons with substitution by radioisotopes or combinations of radioisotopes for radioassay detection or suitable groups for fluorescence detection after separation;

for electrophoresis: see above, particularly functionalized charged molecules, e.g. cationic or anionic, particularly organic or inorganic acid groups, where the molecule may be further modified by having a detectable radioisotope or fluorescer for detection by electrophoresis;

for mass spectroscopy: see above, particularly different mass numbers due to different isotopes, different numbers of the same functionality or different functionalities, different members of a homologous series or combinations thereof.

The separation of tags from one another may involve individual techniques or combinations of techniques, e.g. chromatography and electrophoresis; gas chromatography and mass spectroscopy; etc.

The tags of the present invention will have a property which allows detection at very low levels, usually not greater than nanomole, preferably picomole or less, more preferably femtomole or less, in the presence of other compounds which may be present at significantly higher levels. For this reason, specific atomic substitutions

-56-

may be used to render the labels easily detectable. Such substitutions include:

- 5 (a) substitution by electronegative elements, e.g. fluorine or chlorine, for electron capture detection in conjunction with capillary GC or negative ion mass spectroscopy detection;
- (b) substitution by an uncommon element (excluding C, H, and O) for atomic emission detection in conjunction with capillary GC;
- 10 (c) substitution by several uncommon elements for atomic emission detection to determine the ratio between the elements;
- (d) substitution by a radioactive element, e.g.  $^3\text{H}$ , for detection by autoradiography or scintillation counting in conjunction with LC, TLC or electrophoresis;
- 15 (e) substitution by a multiplicity of radioactive elements having differing emissions, e.g.  $^3\text{H}$  and  $^{14}\text{C}$ , for detection by autoradiography or scintillation counting to determine the ratio of the different radioactive elements.

20 For single-element substitution (a., b., d. above) a separable mixture of A tags whose simple presence or absence can be detected would encode up to  $2^A$  different syntheses. For multiple-element substitution (see, c. and  
25 e. above) a separable mixture of A tags each having B distinguishable states (e.g., different  $^3\text{H}/^{14}\text{C}$  ratios, different Si/Sn ratios) would be able to encode for up to  $B^A$  different syntheses.

30 A wide variety of isotopes exist, where the presence or ratio of isotopes may provide information as to stage and choice. The isotopes may be radioactive or non-radioactive. Isotopes of particular interest include deuterium, tritium,  $^{14}\text{C}$ ,  $^{32}\text{P}$ ,  $^{131}\text{I}$ , etc.

35 By employing mixtures of isotopically-modified compounds, one can greatly expand the information obtained from a

-57-

single tag compound which is only distinguished by the presence of isotopes. For example, one could prepare a mixture of ratios of hydrogen to deuterium, where the various ratios could differ by as little as 10% each.

5

By replacing hydrogens with another atom, such as fluorine, one would then have a varying mixture of hydrogens, deuteriums and fluorines, providing for a large number of different differentiable tags.

10

Other groups that may be involved could be aromatic rings, which are differentially substituted, as to position and functionality. Thus, by having substituted benzene rings, where the position of the substitution and the nature of the substitution can be determined, one can provide for a plurality of molecules which can be distinguished and can provide for both stage and choice information. For example, if  $C$  were constant one could detect and discriminate through the substitution pattern on E when E is a polyhalogenated aromatic ring.

20

There is also the possibility to use fluorescent tags. While fluorescent tags alone may not be sufficient to define a significant number of stages with a significant number of choices, as referred to above, by providing for means for separating the fluorescent tagging molecules based on variations in  $C$  or  $C'$ , one can individually detect the tags by their fluorescence.

25

The mixture of tags associated with a particular bead may be detached and subject to an initial separation, where it is desirable to detect each of the tags separately. Once the group of tags has been separated, each of the tags may then be analyzed based on its particular functionalities and distinctive properties. Various techniques which may be used to detect the particular tags include autoradiography or scintillation counting, electron

35

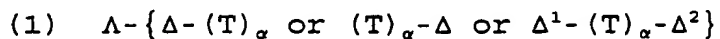
-58-

capture detection, negative or positive ion mass spectroscopy, infrared spectroscopy, ultraviolet spectroscopy, electron spin resonance spectroscopy, fluorescence, and the like.

5

Another composition may have at least 6 different markers being associated in a kit or in a common medium, each marker having a distinguishable moiety which is substantially chemically inert, differing from each other in molecular weight, said markers of the formula:

10



15

where  $\Lambda$  is a linking group which has a functionality for bonding to a solid support and a functionality for detachment from the solid support, which may be included in the functionality for bonding to the solid support;

20

$\Delta$  is a distinguishing group, which allows for the distinguishing by a physical characteristic of each of the markers from each of the other markers by other than fluorescence, so as to provide a set of markers which allows for coding a multistep synthetic procedure, and includes any remaining functional group after detachment, which was previously associated with the linked group;

25

$\Delta^1$  and  $\Delta^2$  are portions of the distinguishing group, which together define a distinguishing group and when joined together come within the definition of  $\Delta$ ;

30

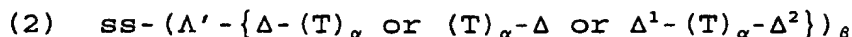
$T$  is a detectable group, which when attached to the distinguishing group allows for the low level detection of the marker, where the detectable group may be present on the marker in the kit or may be added later to the distinguishing group and if attached to the linking group, includes any remaining functional group after detachment, which was previously associated with the linking group;

35



-59-

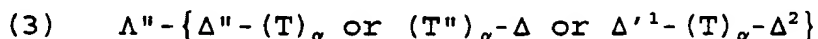
and  $\alpha$  is 0 or 1, indicating that the detectable group may or may not be present;



5

where all of the symbols have been defined previously, except as follows: ss is a solid support;  $\Delta'$  is a linking group covalently bonded to ss; and  $\beta$  is an integer as to each solid support and is at least six and usually not more than about 30;

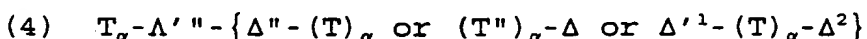
10



15

where all of the symbols have been defined previously, except as follows:  $\Delta''$  is hydrogen or the residue of the linking group after photolytic cleavage, elimination or other chemical reaction which results in detachment from the solid support;  $\Delta''$  or  $\Delta'^1$  is  $\Delta$  or  $\Delta^1$  or a modified  $\Delta$  or  $\Delta^1$ , respectively, as a result of the detachment of the marker from the solid support;  $T''$  is T or a modified T as a result of the detachment of the marker from the solid support;

20



25

where all of the symbols have been defined previously and  $\Lambda'$  is a bond or the remaining portion of the linker group after attachment to T; with the additional limitation that only one  $\alpha$  is 1.

30

### Assays

To determine the characteristic of interest of the product, a wide variety of assays and techniques may be employed.

35

Frequently, in screening the beads, one will use either single beads or mixtures of beads and determine whether

-60-

the bead or mixtures show activity. Thus, the mixtures may involve 10, 100, 1000 or more beads. In this way, large groups of compounds may be rapidly screened and segregated into smaller groups of compounds.

5

One technique is where one is interested in binding to a particular biomolecule such as a receptor. The receptor may be a single molecule, a molecule associated with a microsome or cell, or the like. Where agonist activity is of interest, one may wish to use an intact organism or cell, where the response to the binding of the subject product may be measured. In some instances, it may be desirable to detach the product from the bead, particularly where physiological activity by transduction of a signal is of interest. Various devices are available for detecting cellular response, such as a microphysiometer, available from Molecular Devices, Redwood City, CA. Where binding is of interest, one may use a labeled receptor, where the label is a fluorescer, enzyme, radioisotope, or the like, where one can detect the binding of the receptor to the compound on the bead. Alternatively, one may provide for an antibody to the receptor, where the antibody is labeled, which may allow for amplification of the signal and avoid changing the receptor of interest, which might affect its binding to the product of interest. Binding may also be determined by displacement of a ligand bound to the receptor, where the ligand is labeled with a detectable label.

In some instances, one may be able to carry out a two-stage screen, whereby one first uses binding as an initial screen, followed by biological activity with a viable cell in a second screen. By employing recombinant techniques, one can greatly vary the genetic capability of cells. One can then produce exogenous genes or exogenous transcriptional regulatory sequences, so that binding to a surface membrane protein will result in an observable

-61-

signal, e.g. an intracellular signal. For example, one may introduce a leuco dye into the cell, where an enzyme which transforms the leuco dye to a colored product, particularly a fluorescent product, becomes expressed upon appropriate binding to a surface membrane, e.g.  $\beta$ -galactosidase and digalactosidylfluorescein. In this manner, by associating a particular cell or cells with a particular particle, the fluorescent nature of the cell may be determined using a FACS, so that particles carrying active compounds may be identified. Various techniques may be employed to ensure that the particle remains bound to the cell, even where the product is released from the particle. For example, one may use antibodies on the particle to a surface membrane protein, one may link avidin to the surface of the cell and have biotin present on the particle, etc.

Assays may be performed stagewise using individual particles or groups of particles or combinations thereof. For example, after carrying out the combinatorial syntheses, groups of about 50 to 10,000 particles may be segregated in separate vessels. In each vessel, as to each particle a portion of the product bound to the particle is released. The fractional release may be as a result of differential linking of the product to the particle or using a limited amount of a reagent, condition or the like, so that the average number of product molecules released per particle is less than the total number of product molecules per particle. One would then have a mixture of products in a small volume. The mixture could then be used in an assay for binding, where the binding event could be inhibition of a known binding ligand binding to a receptor, activation or inhibition of a metabolic process of a cell, or the like. Various assay conditions may be used for the detection of binding activity as will be described subsequently. Once a group is shown to be active, the individual particles may then

-62-

be screened, by the same or a different assay. One could of course, have a three- or four-stage procedure, where large groups are divided up into smaller groups, etc. and finally single particles are screened. In each case, portions of the products on the particles would be released and the resulting mixture used in an appropriate assay. The assays could be the same or different, the more sophisticated and time consuming assays being used in the later or last stage.

One may also provide for spatial arrays, where the particles may be distributed over a honeycomb plate, with each well in the honeycomb having 0 or 1 particle.

The subject methodology may be used to find chemicals with catalytic properties, such as hydrolytic activity, e.g. esterase activity. For this purpose one might embed beads in a semisolid matrix surrounded by diffusible test substrates. If the catalytic activity can be detected locally by processes that do not disturb the matrix, for example, by changes in the absorption of light or by detection of fluorescence due to a cleaved substrate, the beads in the zone of catalytic activity can be isolated and their labels decoded.

Instead of catalytic activity, compounds with inhibitory or activating activity can be developed. Compounds may be sought that inhibit or activate an enzyme or block a binding reaction. To detect beads that inhibit an enzyme, which beads have an attached product with this desirable property, it is advantageous to be able to release the products from the beads, enabling them to diffuse into a semisolid matrix or onto a filter where this inhibition, activation or blocking can be observed. The beads that form a visualized or otherwise detectable zone of inhibition, activation or blocking can then be picked and the tags decoded. In this case it is necessary that a

-63-

portion of the synthesized products be attached to the beads by cleavable linkages, preferably a photolabile linkage, while a portion of the tags remain attached to the bead, releasable after picking by a different means than before.

A dialysis membrane may be employed where a layer of beads is separated from a layer of radiolabeled ligand/receptor pair. The bead layer could be irradiated with ultraviolet light and the product released from the bead would diffuse to the pair layer, where the radiolabeled ligand would be released in proportion to the affinity of the compound for the receptor. The radiolabeled ligand would diffuse back to the layer of beads. Since the radiolabel would be proximal to the bead, beads associated with radioemission would be analyzed.

Of particular interest is finding products that have biological activity. In some applications it is desirable to find a product that has an effect on living cells, such as inhibition of microbial growth, inhibition of viral growth, inhibition of gene expression or activation of gene expression. Screening of the compounds on the beads can be readily achieved, for example, by embedding the beads in a semisolid medium and the library of product molecules released from the beads (while the beads are retained) enabling the compounds to diffuse into the surrounding medium. The effects, such as plaques within a bacterial lawn, can be observed. Zones of growth inhibition or growth activation or effects on gene expression can then be visualized and the beads at the center of the zone picked and analyzed.

One assay scheme will involve gels where the molecule or system, e.g. cell, to be acted upon may be embedded substantially homogeneously in the gel. Various gelling agents may be used such as polyacrylamide, agarose,

- 64 -

5     gelatin, etc. The particles may then be spread over the gel so as to have sufficient separation between the particles to allow for individual detection. If the desired product is to have hydrolytic activity, a substrate is present in the gel which would provide a fluorescent product. One would then screen the gel for fluorescence and mechanically select the particles associated with the fluorescent signal.

10     One could have cells embedded in the gel, in effect creating a cellular lawn. The particles would be spread out as indicated above. Of course, one could place a grid over the gel defining areas of one or no particle. If cytotoxicity were the criterion, one could release the  
15     product, incubate for a sufficient time, followed by spreading a vital dye over the gel. Those cells which absorbed the dye or did not absorb the dye could then be distinguished.

20     As indicated above, cells can be genetically engineered so as to indicate when a signal has been transduced. There are many receptors for which the genes are known whose expression is activated. By inserting an exogenous gene into a site where the gene is under the transcriptional  
25     control of the promoter responsive to such receptor, an enzyme can be produced which provides a detectable signal, e.g. a fluorescent signal. The particle associated with the fluorescent cell(s) may then be analyzed for its reaction history.

30

#### Libraries and Kits

For convenience, libraries and/or kits may be provided. The libraries would comprise the particles to which a library of products and tags have been added so as to  
35     allow for screening of the products bound to the bead or the libraries would comprise the products removed from the bead and grouped singly or in a set of 10 to 100 to

-65-

1000 members for screening. The kits would provide various reagents for use as tags in carrying out the library syntheses. The kits will usually have at least 4, usually 5, different compounds in separate containers, more usually at least 10, and may comprise at least  $10^2$  different separated organic compounds, usually not more than about  $10^2$ , more usually not more than about 36 different compounds. For binary determinations, the mode of detection will usually be common to the compounds associated with the analysis, so that there may be a common chromophore, a common atom for detection, etc. Where each of the identifiers is pre-prepared, each will be characterized by having a distinguishable composition encoding choice and stage which can be determined by a physical measurement and including groups or all of the compounds sharing at least one common functionality.

Alternatively, the kit can provide reactants which can be combined to provide the various identifiers. In this situation, the kit will comprise a plurality of separated first functional, frequently bifunctional, organic compounds, usually four or more, generally one for each stage of the synthesis, where the functional organic compounds share the same functionality and are distinguishable as to at least one determinable characteristic. In addition, one would have at least one, usually at least two, second organic compounds capable of reacting with a functionality of the functional organic compounds and capable of forming mixtures which are distinguishable as to the amount of each of said second organic compounds. For example, one could have a glycol, amino acid, or a glycolic acid, where the various bifunctional compounds are distinguished by the number of fluorine or chlorine atoms present, to define stage, and have an iodomethane, where one iodomethane has no radioisotope, another has  $^{14}\text{C}$  and another has one or more  $^3\text{H}$ . By using two or more of the iodomethanes, one could

-66-

provide a variety of mixtures which could be determined by their radioemissions. Alternatively, one could have a plurality of second organic compounds, which could be used in a binary code.

5

As indicated previously one could react the tags after release with a molecule which allows for detection. In this way the tags could be quite simple, having the same functionality for linking to the particle as to the detectable moiety. For example, by being linked to a hydroxycarboxyl group, a hydroxyl group would be released, which could then be esterified or etherified with the molecule which allows for detection. For example, by using combinations of fluoro- and chloroalkyl groups, in the binary mode, the number of fluoro and/or chloro groups could determine choice, while the number of carbon atoms would indicate stage.

Groups of compounds of particular interest include distinguishing groups such as  $\text{C}$  joined to a substituted ortho-nitrobenzyloxy group, indanyloxy or fluorenyloxy group, or other group which allows for photolytic or other selective cleavage. The distinguishing group may be an alkylene group of from 2 to 20 carbon atoms, polyalkyleneoxy, particularly alkyleneoxy of from 2 to 3 carbon atoms, cycloalkyl group of from 4 to 8 carbon atoms, haloalkyl group, particularly fluoroalkyl of from 2 to 20 carbon atoms, one or more aromatic rings and the like, where the distinguishing group provides for the discrimination between the various groups, by having different numbers of units and/or substituents.

Individual particles or a plurality of particles could be provided as articles of commerce, particularly where the particle(s) have shown a characteristic of interest. Based on the associated tags, the reaction history may be decoded. The product may then be produced in a large

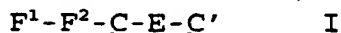


-67-

synthesis. Where the reaction history unequivocally defines the structure, the same or analogous reaction series may be used to produce the product in a large batch. Where the reaction history does not unambiguously define the structure, one would repeat the reaction history in a large batch and use the resulting product for structural analysis. In some instances it may be found that the reaction series of the combinatorial chemistry may not be the preferred way to produce the product in large amounts.

An embodiment of this invention is a kit comprising a plurality of separated organic compounds, each of the compounds characterized by having a distinguishable composition, encoding at least one bit of different information which can be determined by a physical measurement, and sharing at least one common functionality. A preferred embodiment is a kit comprising at least 4 different functional organic compounds.

More preferred is a kit wherein said functional organic compounds are of the formula:



and the formula:



where  $F^1-F^2$  is a linker which allows for attachment to and detachment from a solid particle; and  $\underline{C}-E-\underline{C}'$  is a tag member which can be determined by a physical measurement, especially wherein said functional organic compounds differ by the number of methylene groups and/or halogens, nitrogens or sulfurs present.

Also preferred is a kit wherein the  $\underline{C}-E-\underline{C}'$  portion is removed photochemically or a kit wherein the  $\underline{C}-E-\underline{C}'$  portion is removed oxidatively, hydrolytically, thermolytically, or reductively.

-68-

In one embodiment the invention is a composition comprising at least 6 different components, each component having a distinguishable moiety. The components may be characterized by each moiety being substantially chemically stable or inert and having an identifiable characteristic different from each of the other moieties. Each moiety is joined to a linking group having an active functionality capable of forming a covalent bond through a linking group to individually separable solid surfaces, or joined to a group which is detectable at less than 1 nanomole, with a proviso that when the moieties are joined to the linking group, the components are physically segregated. Preferably, the solid supports are beads. In one embodiment each component comprises molecules of different compounds bound to individual separable solid surfaces, wherein the molecules on the solid surfaces. Preferably, the moieties of the invention define an homologous series and/or a series of substitutions on a core molecule.

The invention herein is also directed to a compound library comprising at least one hundred unique solid supports. In this compound library each solid support has (1) an individual compound bound to the solid support as a major compound bound to the support; and (2) a plurality of tags e.g. tags incapable of being sequenced, where the tags are individual tag molecules which are physically distinguishable in being physically separable and are substituted so as to be detectable at less than about a nanomole or have a functional group for bonding to a substituent which is detectable at less than about a nanomole. Preferably, in the compound library each solid support has at least about 6 tags. In another embodiment, in the compound library the tags define a binary code encoding the synthetic protocol used for the synthesizing of the compound on the solid support.

-69-

This invention also provides a method for determining a synthetic protocol encoded by separable physically different tags in a series and defining a binary code. In this method at least two tags are employed to define each stage of the synthetic protocol, there being at least six tags. The step of the method comprises separating tags by means of their physical differences and detecting the tags. The synthetic protocol is defined a binary code of different tags.

10

Compounds of this invention may be useful as analgesics and/or for the treatment of inflammatory disease, especially in the case of the azatricyclics acting as antagonists of the neurokinin 1/bradykinin receptor. Members of the benzediazopine library may be useful as a muscle relaxant and/or tranquilizer and/or as a sedative. Members of the 23.5 Million Mixed Amide Library (Example 6) may be of use in the treatment of hypertension or Raynaud's syndrome by acting as endothelin antagonists.

20

#### EXAMPLE 1

##### PEPTIDE LIBRARY

In order to encode up to  $10^9$  different syntheses, one could prepare 30 different identifiers which carry individual tags capable of being separated one from another by capillary GC. For encoding a smaller number of syntheses, fewer identifiers would be used. The tags would normally be prepared from commercially-available chemicals as evidenced by the following illustration.  $\omega$ -Hydroxyalkenes-1, where the number of methylene groups would vary from 1 to 5, would be reacted with an iodoperfluoroalkane, where the number of  $\text{CF}_2$  groups would be 3, 4, 6, 8, 10, and 12. By employing a free-radical catalyst, the iodoperfluorocarbon would add to the double bond, where the iodo group could then be reduced with hydrogen and a catalyst or a tin hydride. In this manner, 30 different tags could be prepared. The chemical

35

-70-

procedure is described by Haszeldine and Steele, *J. Chem. Soc. (London)*, 1199 (1953); Brace, *J. Fluor. Chem.*, 20, 313 (1982). The highly fluorinated tags can be easily detected by electron capture, have different GC retention times, so that they are readily separated by capillary GC, are chemically inert due to their fluorinated, hydrocarbon structure and each bears a single hydroxyl functional group for direct or indirect attachment to particles.

Before attachment to compound precursors, the tags (referred to as T1-T30) would be activated in a way which is appropriate for the chemical intermediates to be used in the combinatorial synthesis. By appropriate it is intended that a functionality would be added which allows for ready attachment by a chemical bond to a compound precursor or to the bead matrix itself. The activation process would be applied to each of the 30 different tags and allow these tags to be chemically bound, either directly or indirectly, to intermediates in the combinatorial compound synthesis. For example, a carboxy derivative could be used for coupling and upon activation the resulting carboxy group would bond to the particle.

In the case of a combinatorial synthesis of a peptidic compound or other structure made of amide-linked organic fragments, the encoding process could consist of addition of a carboxylic acid-equipped linker. For example, the tag would be coupled to the tert-butyl ester of o-nitro-p-carboxybenzyl bromide in the presence of sodium hydride. The ester would then be hydrolyzed in dilute trifluoroacetic acid.

Activated identifiers would be coupled to intermediates at each stage in the combinatorial compound synthesis. The ortho-nitrobenzyl ether part of the activated identifiers is used to allow photochemical detachment of the tags after completing the combinatorial synthesis and selecting

-71-

the most desirable compounds. The detached tags would then be decoded using capillary GC with electron capture detection to yield a history of the synthetic stages used to prepare the compound selected.

5

While there is an almost unlimited set of chemical stages and methods which could be used to prepare combinatorial libraries of compounds, we will use coupling of  $\alpha$ -amino acids to make a combinatorial library of peptides as an example of an application of the encoding methodology. In this example, we will describe preparation of a library of pentapeptides having all combinations of 16 different amino acids at each of the five residue positions. Such a library would contain  $16^5$  members. To uniquely encode all members of this library, 20 detachable tags (T1-T20) as described above would be required.

To prepare the encoded library, we would begin with a large number ( $>10^6$ ) of polymer beads of the type used for Merrifield solid phase synthesis and functionalized by free amino groups. We would divide the beads into 16 equal portions and place a portion in each of 16 different reaction vessels (one vessel for each different  $\alpha$ -amino acid to be added). We would then add a small portion (e.g., 1 mol%) of identifiers to each of the amino acid derivatives (e.g., Fmoc amino acids) to be coupled in the first stage of the combinatorial synthesis. The specific combination of the tags incorporated into the identifiers added would represent a simple binary code which identifies the amino acid used in the first stage of synthesis. The 16 amino acids added would be indicated by numbers 1-16 and any such number could be represented chemically by combinations of the first four tags (T1-T4). In tables 2 and 3, a typical encoding scheme is shown in which the presence or absence of a tag is indicated by a 1 or a 0, respectively. The letter T may represent

-72-

either the the tag or the identifier incorporating that tag.

5     **Table 2.** A typical encoding scheme.

	Amino Acid added in first stage	T4	T3	T2	T1
10	Number 1 (e.g., glycine)	0	0	0	0
	Number 2 (e.g., alanine)	0	0	0	1
	Number 3 (e.g., valine)	0	0	1	0
	Number 4 (e.g., serine)	0	0	1	1
	Number 5 (e.g., threonine)	0	1	0	0
15					
	Number 16 (e.g., tryptophan)	1	1	1	1

20     We would then carry out a standard dicyclohexyl-carbodiimide (DCC) peptide coupling in each of the 16 vessels using the Fmoc amino acids admixed with small amounts of the encoding activated identifiers as indicated above. During the couplings, the amino acids as well as  
25     small amounts (e.g., 1%) of the identifiers would become chemically bound to intermediates attached to the beads.

Next the beads would be thoroughly mixed and again separated into 16 portions. Each portion would again be  
30     placed in a different reaction vessel. A second amino acid admixed with appropriate new activated identifiers (T5-T8) would be added to each vessel and DCC coupling would be carried out as before. The particular mixture of

-73-

the incorporated tags (T5-T8) would again represent a simple binary code for the amino acid added in this, the second stage of the combinatorial synthesis.

5     **Table 3.** A typical encoding scheme.

	Amino Acid added in second stage	T8	T7	T6	T5
	Number 1 (e.g., glycine)	0	0	0	0
10	Number 2 (e.g., alanine)	0	0	0	1
	Number 3 (e.g., valine)	0	0	1	0
	Number 4 (e.g., serine)	0	0	1	1
	Number 5 (e.g., threonine)	0	1	0	0
15					
	Number 16 (e.g., tryptophan)	1	1	1	1

After the 16 couplings of stage 2 are complete, the beads would be again mixed and then divided into 16 new portions for the third stage of the synthesis. For the third stage, T9-T12 would be used to encode the third amino acid bound to the beads using the same scheme used for stages 1 and 2. After the third couplings, the procedure would be repeated two more times using the fourth amino acids with T13-T16 and the fifth amino acids with T17-T20 to give the entire library of 1,048,576 different peptides bound to beads.

Although the above beads would be visually indistinguishable, any bead may be chosen (e.g., by selecting based on the interesting chemical or biological

-74-

properties of its bound peptide or other target molecule) and its synthetic history may be learned by detaching and decoding the associated tags.

5 The precise method used to detach tags will depend upon the particular linker used to chemically bind it to intermediates in the combinatorial synthesis of the target compound. In the example above, the *ortho*-nitrobenzyl carbonate linkages, which are known to be unstable to  
10 ~300 nm light (Ohtsuka, et al., *J. Am. Chem. Soc.*, 100, 8210 [1978]), would be cleaved by photochemical irradiation of the beads. The tags would then diffuse from the beads into free solution which would be injected into a capillary gas chromatograph (GC) equipped with a  
15 sensitive electron capture detector. Since the order in which the tags (T1-T20) emerged from the GC and their retention times under standard conditions were previously determined, the presence or absence of any of T1-T20 would be directly determined by the presence or absence of their  
20 peaks in the GC chromatogram. If 1 and 0 represent the presence and absence respectively of peaks corresponding to T1-T20, then the chromatogram can be taken as a 20-digit binary number which can uniquely represent each possible synthesis leading to each member of the peptide  
25 library. The use of halocarbon tags which are safe, economical and detectable at subpicomole levels by electron capture detection makes this capillary GC method a particularly convenient encoding scheme for the purpose.



-75-

As an example of using the encoding scheme for the pentapeptide library above, a particular bead is irradiated with light to detach the tags, the solubilized labels injected into a capillary GC and the following chromatogram obtained ("Peak" line):

Label	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	GC Inject
Peak																					
Binary	1	1	1	1	0	1	0	0	0	0	1	1	0	0	0	1	0	0	1	0	
Stage	----5-----				----4-----				----3-----				----2-----				----1-----				
AA	Tryptophan				Threonine				Serine				Alanine				Valine				

The "Label" line diagrams the GC chromatogram where T20-T1 peaks (|) are to be found (note the injection is given on the right and the chromatogram reads from right to left). The "Peak" line represents the presence of labels (T20-T1) as peaks in the chromatogram. The "Binary" line gives presence (1) or absence (0) of peaks as a binary number. The "Stage" line breaks up the binary number into the five different parts encoding the five different stages in the synthesis. Finally, the "AA" line gives the identity of the amino acid which was added in each stage and was given by the binary code in the "Binary" line above.

-76-

## EXAMPLE 2

RADIO-LABELED TAGS

In the next illustration, the tags employed are monomethylethers of linear alkyl- $\alpha,\omega$ -diols. The diol  
5 would have  $N + 2$  carbon atoms, where  $N$  designates the stage. The methyl group would be a radiolabeled reagent which would have any of a variety of  $^3\text{H}/^{14}\text{C}$  ratios from 1/1 to  $m/1$ , where  $m$  is the number of choices. The double radiolabel allows for accurate quantitation of the tritium  
10 present in the tag. By having 10 different alkylene groups and 10 different radioactive label ratios,  $10^{10}$  unique ten-member sets of tags are generated. Tags would be attached by first reacting them with activating agents, e.g. phosgene to form a chloroformate, followed by  
15 reaction with the  $\text{F}^1\text{-F}^2$  component. In this case,  $\text{F}^1\text{-F}^2$  is the o-nitro-p-carboxy-benzyl alcohol protected as the t-butyl ester. Each time a synthetic stage is carried out, the de-esterified identifier is added directly to the bead, which has covalently bonded amine or hydroxyl  
20 groups, to form amides or esters with the acid activated using standard chemistry, e.g., carbodiimide coupling methodology. At the end of the sequential synthesis, the beads are then screened with a variety of receptors or enzymes to determine a particular characteristic. The  
25 beads demonstrating the characteristic may then be isolated, the tags detached and separated by HPLC to give a series of glycol monomethyl ethers which may then be analyzed for radioactivity by standard radioisotope

-77-

identification methods. For example, if the first and second tags to elute from the HPLC column had  $^3\text{H}/^{14}\text{C}$  ratios of 5:1 and 7:1 respectively, then the product which showed activity had been synthesized by reagent number 5 in stage 1 and reagent number 7 in stage 2.

### EXAMPLE 3

#### 2401 Peptide Library

The identifiers employed were 2-nitro-4-carboxybenzyl, O-aryl substituted  $\omega$ -hydroxyalkyl carbonate, where alkyl was of from three to 12 carbon atoms and aryl was (A) pentachlorophenyl, (B) 2,4,6-trichlorophenyl, or (C) 2,6-dichloro-4-fluorophenyl. The tags are designated as NAr, wherein N is the number of methylene groups minus two and Ar is the aryl group. Thus, tag 2A has a butylene group bonded to the pentachlorophenyl through oxygen. The subject tags can be easily detected using electron capture gas chromatography at about 100 fmol.

In the subject analysis, the tagging molecules are arranged in their GC elution order. Thus the tag which is retained the longest on the GC column is designated T1 and is associated with the least significant bit in the binary synthesis code number, the next longest retained tag is called T2 representing the next least significant binary bit, and so on. Using an 0.2mm x 20M methylsilicone capillary GC column, eighteen well-resolved tags were obtained where T1 through T18 corresponded to 10A, 9A, 8A,

-78-

7A, 6A, 5A, 4A, 3A, 6B, 2A, 5B, 1A, 4B, 3B, 2B, 1B, 2C, and IC, respectively.

An encoded combinatorial library of 2401 peptides was prepared. This library had the amino acid sequence N-XXXXEEDLGGGG-bead, where the variable X residues were D, E, I, K, L, Q, or S (single letter code). The 4 glycines served as a spacer between the encoded amino acid sequence and the bead. The combinatorial library included the sequence H<sub>2</sub>N-KLISEEDL, part of the 10 amino acid epitope which is known to be bound by 9E10, a monoclonal antibody directed against the human C-myc gene product. For encoding this library, three binary bits were sufficient to represent the seven alternative reagents for each stage. The code was as follows: 001 = S; 010 = I; 011 = K; 100 = L; 101 = Q; 110 = E; 111 = D.

The library was synthesized by first preparing the constant segment of the library H<sub>2</sub>NEEDLGGGG-bead on 1.5 g of 50-90 $\mu$  polystyrene synthesis beads functionalized with 1.1 meq/g of aminomethyl groups using standard solid phase methods based on t-butyl side-chain protection and Fmoc main chain protection (Stewart and Young, "Solid Phase Peptide Synthesis", 2nd edition, Pierce Chemical Co., 1984). After deprotecting the Fmoc groups with diethylamine, the beads were divided into seven 200 mg fractions and each fraction placed in a different Merrifield synthesis vessel mounted on a single wrist-

-79-

action shaker. The beads in the seven vessels were processed independently as follows (see Table 3-1). The letter T in this example refers to the tag or to the identifier incorporating that tag.

5

TABLE 3-1

10

15

Ves sel No.	Step 1	Step 2	Step 3	Step 4
1	1%T1	DIC, wash	Fmoc(tBu)S, Anh.	Wash
2	1%T2	"	FmocI, Anh.	"
3	1%T1,T2	"	Fmoc(Boc)K, Anh.	"
4	1%T3	"	FmocL, Anh.	"
5	1%T1,T3	"	Fmoc(trityl)Q, Anh.	"
6	1%T2,T3	"	Fmoc(t-butyl)E, Anh.	"
7	1%T1,T2,T3	"	Fmoc(tBu)D, Anh.	"

20

25

In accordance with the above procedure a sufficient amount of the identifiers listed in step 1 were attached via their carboxylic acids using diisopropylcarbodiimide to tag about 1% of the free amino groups on each bead in the corresponding vessel. The remaining free amino groups on each bead were then coupled in step 3 to N-protected amino acid anhydrides. After washing with methylene chloride, isopropanol, and N,N-dimethylformamide, the beads from the seven vessels were combined and thoroughly mixed. At this point the library had seven members.

-80-

After Fmoc deprotection (diethylamine), the beads were again divided into seven vessels and processed as before except that in place of the identifiers used previously, identifiers representing the second stage (T4-6) were used. By repeating the procedure two more times, using identifiers T7-9 and then T10-12 analogously, the entire uniquely encoded library of  $7^4=2401$  different peptides was prepared using only 12 identifiers.

To read the synthesis code from a single selected bead, the bead was first washed four times in a small centrifuge tube with 100  $\mu$ L portions of DMF, and then resuspended in 1  $\mu$ L of DMF in a Pyrex capillary tube. After 2 hrs of photolysis with a Rayonet 350 nm light source, the tags released from the bound identifiers were silylated using about 0.1  $\mu$ L bis-trimethylsilylacetamide and the solution injected into a Hewlett Packard capillary gas chromatograph equipped with an 0.2mm x 20M methylsilicone fused silica capillary column and an electron capture detector. The binary synthesis code of the selected bead was directly determined from the chromatogram of the tags which resulted.

-81-

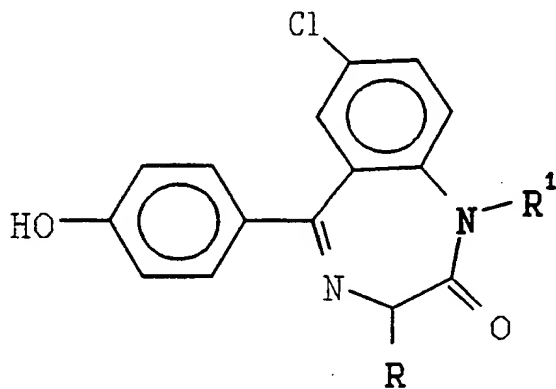
## EXAMPLE 4

Benzodiazepine Library

A combinatorial benzodiazepine library comprising 30 compounds of the formula VIII

5

10



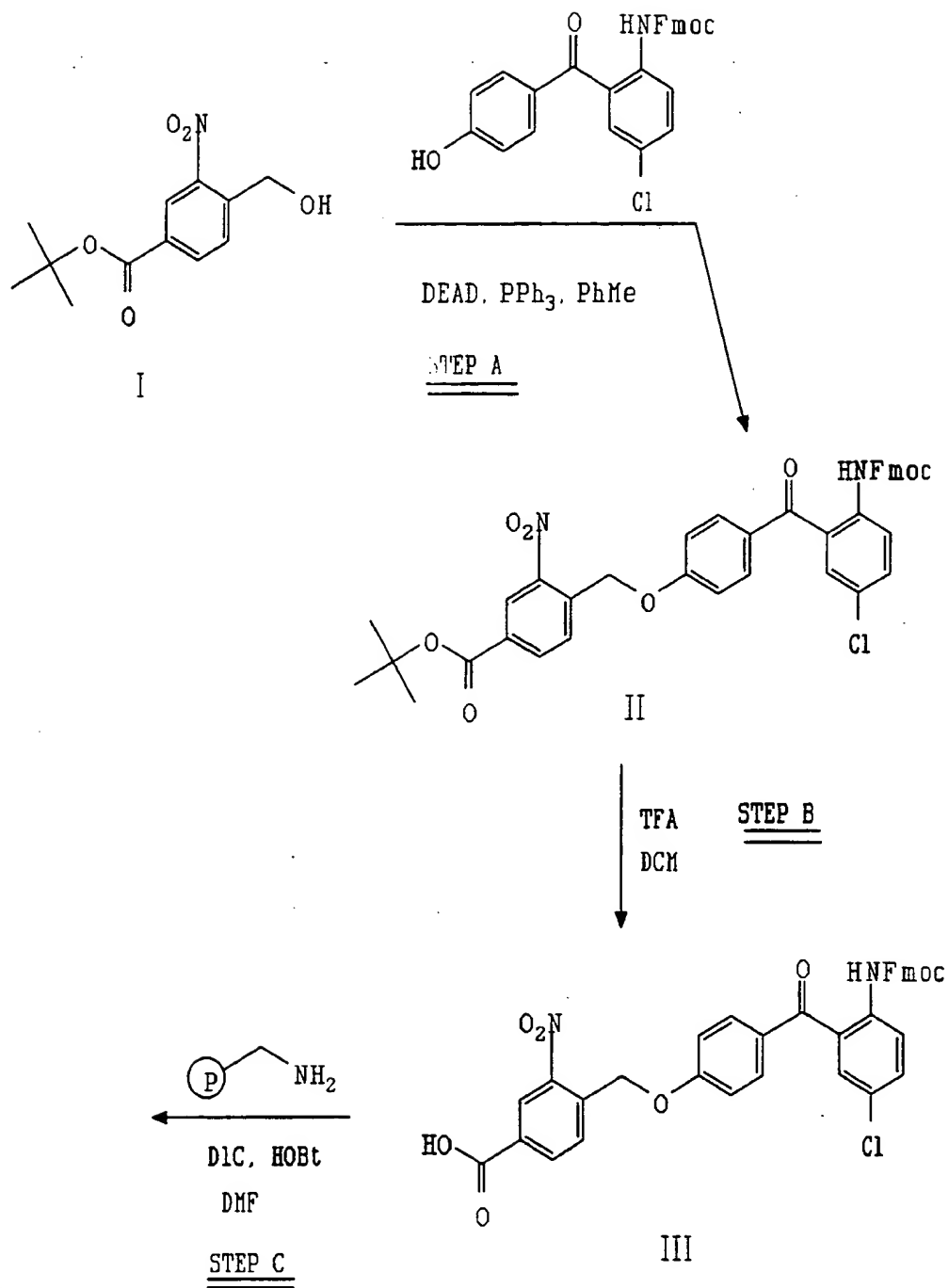
VIII

wherein:

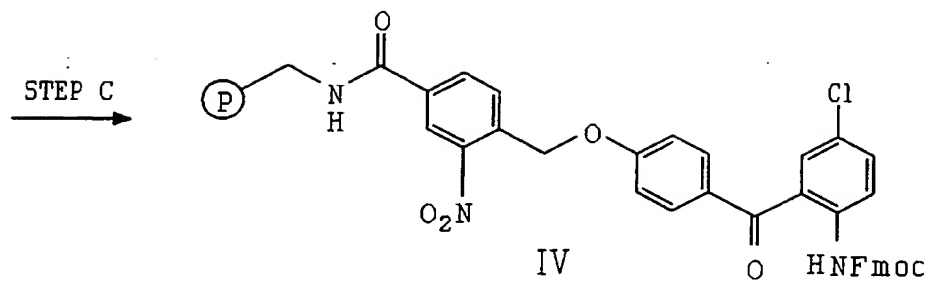
R is CH<sub>3</sub>, CH(CH<sub>3</sub>)<sub>2</sub>, CH<sub>2</sub>CO<sub>2</sub>H, (CH<sub>2</sub>)<sub>4</sub>NH<sub>2</sub>, CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OH, or CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>  
and

15 R¹ is H, CH<sub>3</sub>, C<sub>2</sub>H<sub>5</sub>, CH<sub>2</sub>CH=CH<sub>2</sub>, or CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>  
is constructed per the following scheme.

- 82 -



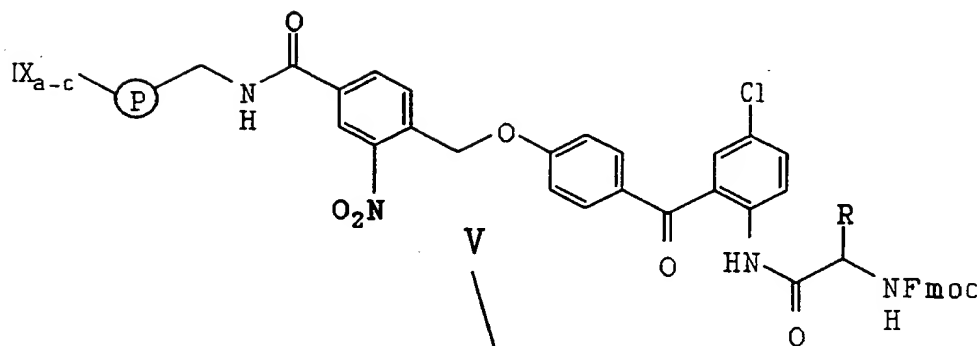
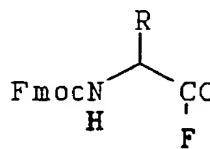




Ⓟ = POLYSTYRENE RESIN

**STEP D**

- 1) TAGS IX<sub>a-c</sub>
- 2) 20% PIPERIDINE IN DMF

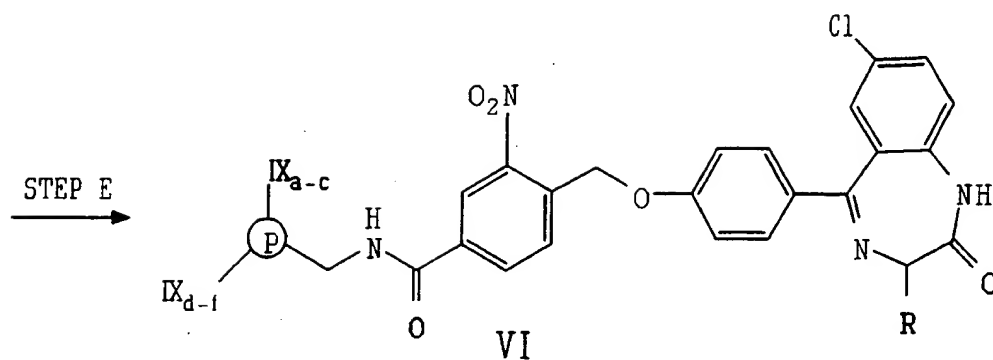


- 1) TAGS IX<sub>d-f</sub>
- 2) 20% PIPERIDINE/DMF
- 3) 5 AcOH/DMF

60 °C

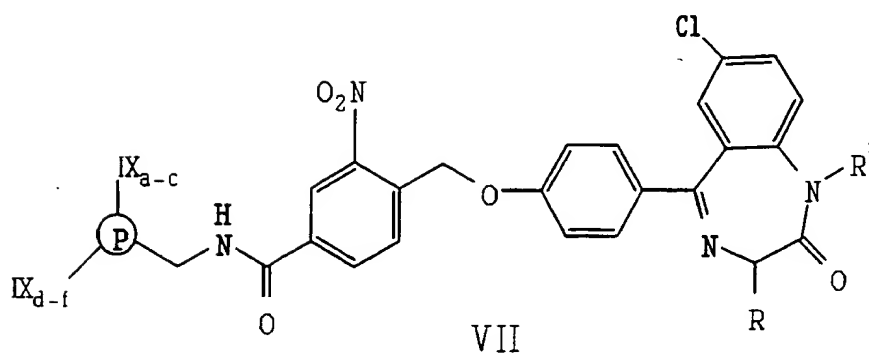
### STEP E

- 84 -



STEP F

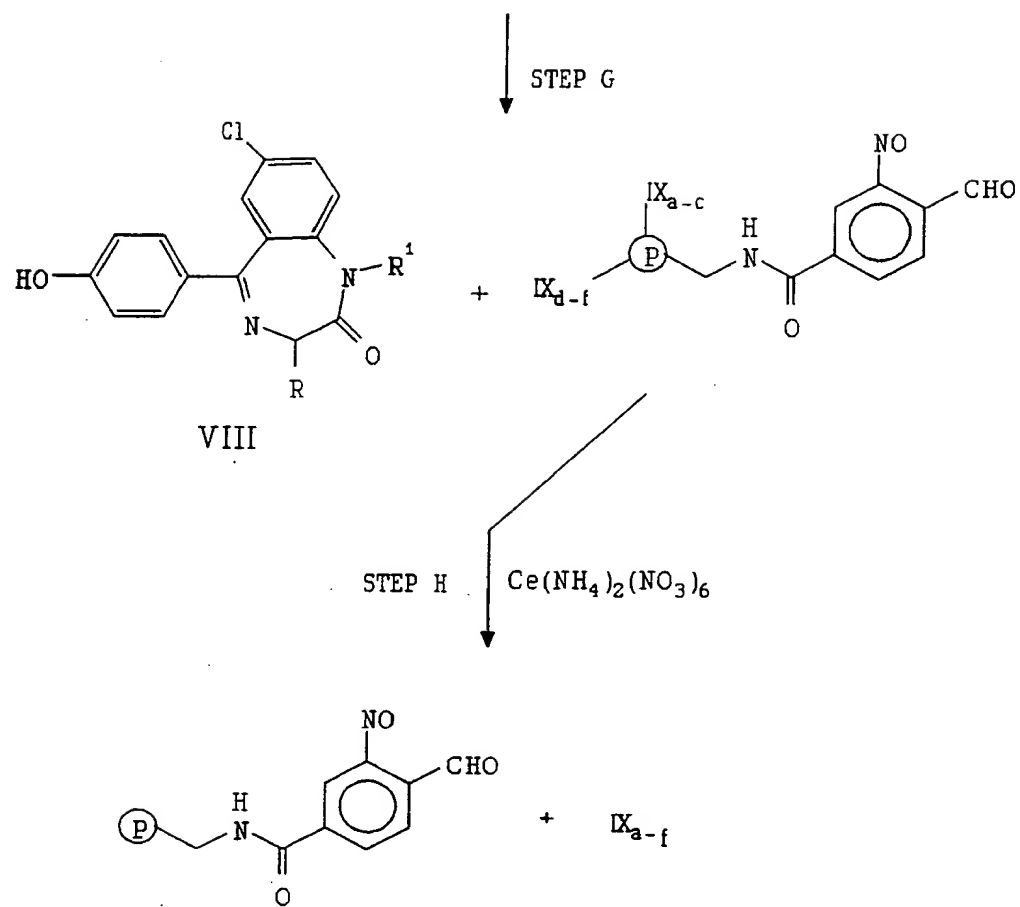
- 1) LITHIATED 5(PHENYLMETHYL)-2-  
OXAZOLIDINONE  
THF,  $-78^{\circ}\text{C}$
- 2)  $\text{R}^1\text{X}$ , DMF  
X=BROMINE OR IODINE
- 3)  $\text{TFA}:\text{H}_2\text{O}:\text{DMETHYLSULFIDE}$   
95:5:10



STEP G

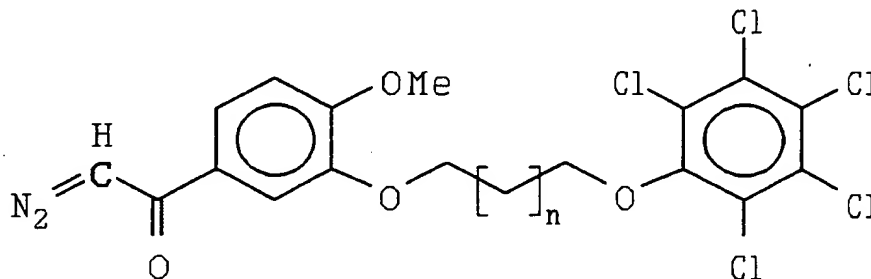
$h\nu$  (350 nm)  
DMF

- 85 -



The benzodiazepines VIII are constructed on polystyrene beads similarly to the method of Bunin and Ellman (JACS, 114, 10997-10998 [1992]) except that a photolabile linker is incorporated between the bead and the benzodiazepine (see steps A, B, and C), thus allowing the benzodiazepine to be removed in step G non-hydrolytically by exposure to U.V. light (350 nm in DMF for 10 minutes to 12 hr). Additionally, binary codes are introduced in steps D and E which allow for a precise determination of the reaction sequence used to introduce each of the 6 R's and 5 R<sup>1</sup>'s. After removal of the tags according to step H and analysis by electron capture detection following GC separation, the nature of the individual R and R<sup>1</sup> groups is determined.

Steps D, E, and F essentially follow the procedure of Bunin and Ellman, but also include the incorporation of identifiers IXa-c in step D and IXd-f in Step E. The identifiers are all represented by Formula IX,



IX

- 87 -

wherein:

$IX_a$  indicates  $n=6$ ;

$IX_b$  indicates  $n=5$ ;

$IX_c$  indicates  $n=4$ ;

5.  $IX_d$  indicates  $n=3$ ;

$IX_e$  indicates  $n=2$ ; and

$IX_f$  indicates  $n=1$ .

The codes for each of R and  $R^1$  are as follows:

10

Table 4-1

15

20

<u>IX</u>	<u>R</u>
a	$CH_3$
b	$CH(CH_3)_2$
a, b	$CH_2CO_2H$
c	$(CH_2)_4NH_2$
a, c	$CH_2-C_6H_4-4-OH$
b, c	$CH_2C_6H_5$
<u>IX</u>	<u><math>R^1</math></u>
d	H
e	$CH_3$
d, e	$C_2H_5$
f	$CH_2CH=CH_2$
d, f	$CH_2C_6H_5$

Step A

To a solution of I (1 equiv) in toluene (conc. = 0.5 M) is added the Fmoc protected 2-amino-5-chloro-4'-hydroxy-benzophenone (1.3 eq) and diethylazodicarboxylate (1.3 eq) and triphenylphosphine (1.3 eq). The mixture is stirred at room temperature for 24 hr. The solvent is removed in vacuo and the residue triturated with ether and filtered and the solvent again removed in vacuo. The resultant product II is purified by chromatography on silica gel.

10

Step B

To a solution of II in DCM (0.2 M) stirring at r.t. is added TFA (3 equiv.) and the solution is allowed to stir for 12 hr. The solution is evaporated to dryness in vacuo and the residue dissolved in DCM, washed once with brine and dried ( $\text{Na}_2\text{SO}_4$ ). Filtration and evaporation of the solvent affords III.

15

Step C

1% DVB (divinylbenzene) cross-linked polystyrene beads ( $50\mu$ ) functionalized with aminomethyl groups (1.1 mEq/g) are suspended in DMF in a peptide reaction vessel (Merrifield vessel). III (2 equiv) and HOBt (3 equiv) in DMF are added and the vessel shaken for 10 min. DIC (3 eq) is added and the vessel is shaken until a negative Ninhydrin test indicates completion of the reaction after 12 hr.

25

-89-

The DMF is removed and the resin washed with additional DMF (x5) and DCM (x5) before drying in vacuo.

Step D

- 5 The dry resin is divided into 6 reaction vessels and is suspended in DCM. The appropriate combinations of identifiers IX<sub>a-c</sub> (see Table 4-1) are added to the flasks and shaken for 1 hr. The Rh(TFA)<sub>2</sub> catalyst (1 mol%) is added to each flask and shaken for an additional 2 hr.
- 10 The flasks are drained and the resin washed with DCM (x5). The resin is then treated with a solution of TFA in DCM (0.01 M) and shaken for 30 min. and then washed again with DCM (x3) followed by DMF (x2). The resin is treated with a 20% solution of piperidine in DMF and shaken for 30 min.
- 15 and is then washed with DMF (x3) and DCM (x3).

- To each flask is added the appropriate Fmoc protected amino acylfluoride (3 equiv) (when required side-chain functional groups are protected as tert-butyl ester (Asp),
- 20 tert-butyl ether (Tyr) or tert-butyloxycarbonyl (Lys)) with 2,6-di-tert-butyl-4-methylpyridine (10 equiv) and the flasks shaken overnight or until a negative Ninhydrin test is achieved. The resin is washed once (DCM) and then the six batches are combined and washed again (DCM, x5) before
- 25 drying in vacuo.

-90-

Step E

The dry resin is divided into five reaction vessels and is suspended in DCM. The appropriate combinations of identifiers IX<sub>d-f</sub> (see Table 4-1) are added to the flasks and shaken for 1 hr. The Rh(TFA)<sub>2</sub> catalyst (1 mol%) is added to each flask and shaken for an additional 2 hr. The flasks are drained and the resin washed with DCM (x5). The resin is then treated with a solution of TFA in DCM (0.01 M) and shaken for 30 min. and is then washed with DMF (x3) and DCM (x3).

To each flask is added a solution of 5% acetic acid in DMF and the mixtures are heated to 60°C and shaken overnight. The solvent is drained and then the resin washed with DMF (x5).

Step F

Each batch of resin is suspended in THF and the flasks are cooled to -78°C. To each flask is added a solution of lithiated 5-(phenylmethyl)-2-oxazolidinone (2 equiv) in THF and the mixtures are shaken at -78°C for 1 hr. The appropriate alkylating agent (Table 4-2) (4 equiv) is then added to each reaction flask followed by a catalytic amount of DMF. The vessels are allowed to warm to ambient temperature and shaken at this temperature for 5 hrs. The solvent is removed by filtration and the resin washed with THF (x1) and then dried in vacuo. The batches of resin are then combined and washed with THF (x2) and DCM (x2)



-91-

and the combined resin is then treated with a 95:5:10 mixture of TFA:water:dimethylsulphide for 2 hrs to remove the side chain protecting groups.

TABLE 4-2

<u>IDENTIFIER</u>	<u>ALKYLATING AGENT</u>
e	H <sub>3</sub> CI
d,e	C <sub>2</sub> H <sub>5</sub> Br
f	BrCH <sub>2</sub> -CH=CH <sub>2</sub>
d,f	BrCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>

Step G

The resultant benzodiazepine can be cleaved from a bead of polystyrene by suspending the bead in DMF and irradiating with U.V. (350 nm) for 12 hrs.

Step H

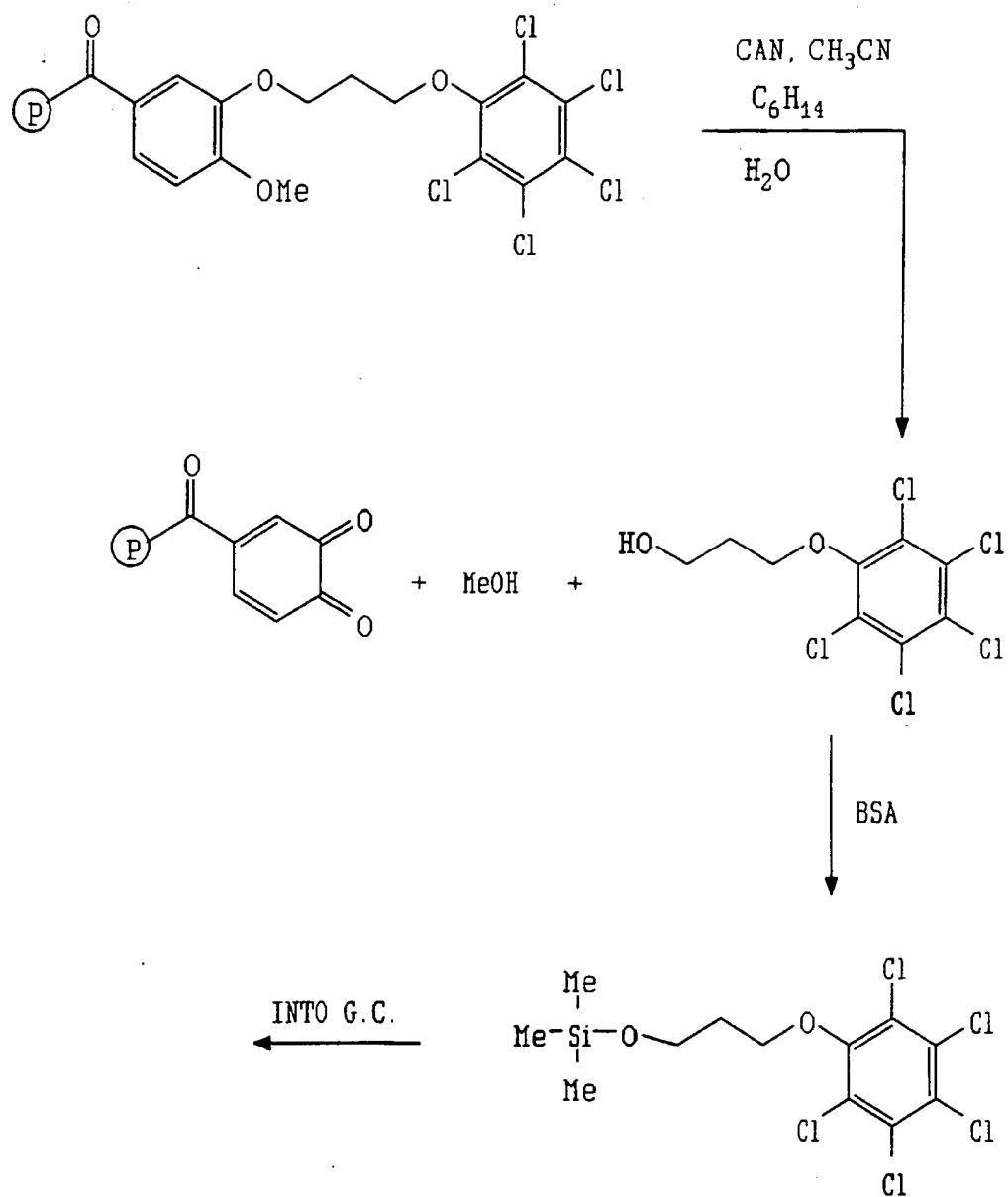
A bead of interest is placed into a glass capillary tube. Into the tube is syringed 1  $\mu$ L of 1M aqueous cerium (IV) ammonium nitrate (CAN) solution, 1  $\mu$ L of acetonitrile and 2  $\mu$ L of hexane. The tube is flame sealed and then centrifuged to ensure that the bead is immersed in the reagents. The tube is placed in an ultrasonic bath and sonicated from 1 to 10 hrs preferably from 2 to 6 hrs.

-92-

The tube is cracked open and  $\approx 1 \mu\text{L}$  of the upper hexane layer is mixed with  $\approx 0.2 \mu\text{L}$  of bis(trimethylsilyl)-acetamide (BSA) prior to injection into the GC and each tag member determined using electron capture detection, as

5 exemplified in the following scheme.

- 93 -



- 94 -

## EXAMPLE 5

117,649 Peptide Library

An encoded library of 117,649 peptides was prepared. This library had the sequence H<sub>2</sub>N-XXXXXXEEDLGGGG-bead, where the  
5 variable residue X was D, E, I, K, L, Q or S. This library was encoded using the 18 tags as defined in Example 3; three binary bits being sufficient to represent the seven amino acids used in each step. The code was: 001=S; 010=I; 011=K; 100=L; 101=Q; 110=E; and 111=D, where 1 indicates  
10 the presence and 0 indicates the absence of a tag.

The constant segment of the library (H<sub>2</sub>NEEDLGGGG-bead) was synthesized on 1.5 g of 50-80  $\mu$  Merrifield polystyrene synthesis beads functionalized with 1.1 mEq/g of  
15 aminomethyl groups using standard solid phase methods based on t-Bu sidechain protection and Fmoc mainchain protection. After deprotecting the N-terminal Fmoc protecting group with diethylamine, the beads were divided into seven 200 mg portions, each portion being placed into  
20 a different Merrifield synthesis vessel mounted on a single wrist-action shaker.

The beads in the seven vessels were processed as in Table 3-1 to attach the sets of identifiers (T1-T3) and the  
25 corresponding amino acid to each portion except that instead of DIC, i-butylchloroformate was used for activation.

-95-

This procedure first chemically attached small amounts of appropriate identifiers via their carboxylic acids to the synthesis beads. This attachment was achieved by activating the linker carboxyl groups as mixed carbonic anhydrides using isobutylchloroformate, and then adding an amount of activated identifier corresponding to 1% of the free amino groups attached to the beads. Thus, about 1% of the free amino groups were terminated for each identifier added. The remaining free amino groups were then coupled in the usual way with the corresponding protected amino acids activated as their symmetrical anhydrides.

After washing, the seven portions were combined and the Fmoc protected amino groups were deprotected by treatment with diethylamine. The beads were again divided into seven portions and processed as before, except that the appropriate identifiers carrying tags T4, T5, and T6 were added to the reaction vessels.

The procedure of dividing, labelling, coupling the amino acid combining and main-chain deprotection was carried out a total of six times using identifiers bearing tags T1-T18, affording an encoded peptide library of 117,649 different members.

25

-96-

Typical Identifier Preparation

To a solution of 8-bromo-1-octanol (0.91 g, 4.35 mmol) and 2,4,6-trichlorophenol (1.03 g, 5.22 mmol) in DMF (5 mL) was added cesium carbonate (1.70 g, 5.22 mmol) resulting  
5 in the evolution of gas and the precipitation of a white solid. The reaction was stirred at 80° C for 2 hrs. The mixture was diluted with toluene (50 mL) and poured into a separatory funnel, washed with 0.5 N NaOH (2x50 mL), 1N HCl (2x50 mL) and water (50 mL) and the organic phase was  
10 dried (MgSO<sub>4</sub>). Removal of the solvent by evaporation gave 1.24 g (87% yield) of tag as a clear oil.

The above tag (0.81 g, 2.5 mmol) was added to a 2 M solution of phosgene in toluene (15 mL) and stirred at  
15 room temperature for 1 hr. The excess phosgene and the toluene were removed by evaporation and the resulting crude chloroformate was dissolved in DCM (5 mL) and pyridine (0.61 mL, 7.5 mmol). tert-Butyl 4-hydroxy-methyl-3-nitrobenzoate (Barany and Albericio, *J. Am. Chem.*  
20 *Soc.*, (1985), 107, 4936-4942) (0.5 g, 1.98 mmol) was added and the reaction mixture stirred at room temperature for 3 hrs. The solution was diluted with ethyl acetate (75 mL) and poured into a separatory funnel. After washing with 1N HCl (3x35 mL), saturated NaHCO<sub>3</sub> (2x35 mL) and brine  
25 (35 mL), the organic phase was dried (MgSO<sub>4</sub>). The solvent was removed by evaporation and the residue purified by chromatography on silica gel (5% to 7.5% ethyl acetate in

-97-

petroleum ether) affording 0.95 g (79% yield) of the identifier tert-butyl ester as a clear oil.

5 Trifluoroacetic acid (3 mL) was added to a solution of the identifier tert-butyl ester (0.95 g, 1.57 mmol) in DCM (30 mL) to deprotect the linker acid (i.e., F<sup>1</sup>-F<sup>2</sup> of Formula I) and the solution was stirred at room temperature for 7 hrs. The mixture was then evaporated to dryness and the residue redissolved in DCM (30 mL). The solution was  
10 washed with brine (20 mL) and the organic phase dried (MgSO<sub>4</sub>). Removal of the solvent by evaporation gave 0.75 g (87% yield) of the identifier (6B) as a pale yellow solid. (Tag nomenclature is the same as in Example 3).

15 Typical Encoded Library Synthesis Step

N $\alpha$ -Fmoc-E(tBu)-E(tBu)-D(tBu)-L-G4-NH-resin was suspended in DMF (20 mL) and shaken for 2 min. After filtering, 1:1 diethylamine:DMF (40 mL) was added to remove the Fmoc protecting groups and the resin was shaken for 1 hr. The  
20 resin was separated by filtration and washed with DMF (2x20 mL, 2 min each); 2:1 dioxane: water (2x20 mL, 5 min each), DMF (3x20 mL, 2 min each), DCM (3 x 20 mL, 2 min each) then dried in vacuo at 25° C. (The resin was found to have 0.4 mmol/g amino groups by picric acid titration  
25 at this stage.)

150 mg Portions of the resin were placed into seven Merrifield vessels and suspended in DCM (5 mL). The

-98-

appropriate identifiers were activated as their acyl carbonates as follows (for the first coupling): T1 (6.6 mg, 0.0098 mmol) was dissolved in anhydrous ether (2 mL) and pyridine (10  $\mu$ L) was added. Isobutyl chloroformate (1.3  $\mu$ L, 0.0096 mmol) was added as a solution in anhydrous ether (0.1 mL). The resulting mixture was stirred at 25° C for 1 hr. during which time a fine white precipitate formed. The stirring was stopped and the precipitate was allowed to settle for 30 min. Solutions of the acylcarbonates of T2 and T3 were prepared in the same way. Aliquots (0.25 mL) of the supernatant solution of activated identifiers were mixed to give the appropriate 3-bit binary tag codes and the appropriate coding mixtures of identifiers were added to each of the seven synthesis vessels. The vessels were shaken in the dark for 12 hrs, and then each was washed with DCM (4x10 mL, 2 min each). A solution of the symmetrical anhydride of an N $\alpha$ -Fmoc amino acid in DCM (3 equivalents in 10 mL) was then added to the corresponding coded batch of resin and shaken for 20 min. 5% N,N-diisopropylethylamine in DCM (1 mL) was added and the mixture shaken until the resin gave a negative Kaiser test.

The resin batches were filtered and combined, and then washed with DCM (4x20 mL, 2 min each), isopropanol (2x20 mL, 2 min each), DCM (4x20 mL, 2 min each). The next cycle of labelling/coupling was initiated by Fmoc deprotection as described above.



-99-

After Fmoc deprotection of the residues in the last position of the peptide, the side chain functionality was deprotected by suspending the resin in DCM (10 mL), adding thioanisole (2 mL), ethanedithiol (0.5 mL) and tri-  
5 fluoroacetic acid (10 mL) then shaking for 1 hr at 25° C. The resin was then washed with DCM (6x20 mL, 2 min each) and dried.

Electron Capture Gas Chromatography Reading of Code

10 A single, selected bead was placed in a Pyrex capillary tube and washed with DMF (5x10  $\mu$ L). The bead was then suspended in DMF (1  $\mu$ L) and the capillary was sealed. The suspended bead was irradiated at 366 nm for 3 hrs to release the tag alcohols, and the capillary tube  
15 subsequently placed in a sand bath at 90° C for 2 hrs. The tube was opened and bis-trimethylsilyl acetamide (0.1 mL) was added to trimethylsilylate the tag alcohols. After centrifuging for 2 min., the tag solution above the bead (1  $\mu$ L) was injected directly into an electron capture  
20 detection, capillary gas chromatograph for analysis. Gas chromatography was performed using a Hewlett Packard Series II Model 5890 gas chromatograph equipped with a 0.2 mmx20 m methylsilicone fused silica capillary column and an electron capture detector. Photolysis reactions were  
25 performed using a UVP "Black Ray" model UVL 56 hand-held 366 nm lamp.

-100-

Antibody Affinity Methods

The anti-C-myc peptide monoclonal antibody 9E10 was prepared from ascites fluid as described in Evans et al., Mol. Cell Biol., 5, 3610-3616 (1985) and Munro and Pelham, Cell, 48, 899-907 (1987). To test beads for binding to 9E10, beads were incubated in TBST [20 mM Tris-HCl (pH 7.5), 500 mM NaCl and 0.05% Tween-20] containing 1% bovine serum albumin (BSA) to block non-specific protein binding sites. The beads were then centrifuged, resuspended in a 1:200 dilution of 9E10 ascites fluid in TBST + 1% BSA and incubated overnight at 4°C. Beads were subsequently washed three times in TBST and incubated for 90 min. at room temperature in alkaline phosphatase-coupled goat antimouse IgG antibodies (Bio-Rad Laboratories), diluted 1:3000 in TBST + 1% BSA. After washing the beads twice in TBST and once in phosphatase buffer (100 mM Tris-HCl, pH 9.5, 100 mM NaCl and 5 mM MgCl<sub>2</sub>), the beads were incubated 1 hr at room temperature in phosphatase buffer containing one one-hundreth part each of AP Color Reagents A & B (Bio-Rad Laboratories). To stop the reaction, the beads were washed twice in 20 mM sodium EDTA, pH 7.4. Solution phase affinities between 9E10 and various peptides were determined by a modification of the competitive ELISA assay described by Harlow et al., Antibodies: a Laboratory Manual, 570-573, Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

-101-

From a 30 mg sample of the combinatorial library of peptides, 40 individual beads were identified which stained on exposure to the anti-C-myc monoclonal antibody. Decoding of these positive-reacting beads established the  
5 ligand's reaction sequence as the myc epitope (EQKLISEEDL) or sequences that differed by one or two substituents among the three N-terminal residues.

#### EXAMPLE 6

##### 10 23,540,625 Mixed Amide Library

The encoding technique was tested further by the preparation of a combinatorial library of 23,540,625 members consisting of peptides and other amide compounds.

15 The synthesis was carried out using 15 different reagents in 5 steps and 31 different reagents in the sixth step. Four identifiers were used to encode each of the 5 steps with 15 reagents and five identifiers were used in the final step with 31 reagents. A label set of 25  
20 identifiers was therefore prepared. 2-Nitro-4-carboxybenzyl, O-aryl substituted  $\omega$ -hydroxyalkyl carbonate identifiers were employed, where the tag components were comprised of an alkyl moiety of from 3 to 12 carbon atoms and the aryl moieties were (A) pentachlorophenyl, (B)  
25 2,4,5-trichlorophenyl, (C) 2,4,6-trichlorophenyl, or (D) 2,6-dichloro-4-fluorophenyl. A set of 25 tags was prepared using appropriate alkyl chains lengths with A, B, C or D, separable using a 0.2 mMx25M methylsilicone GC

-102-

column. The chemical compositions of tags T1-T25 (where T1 represents the tag with the longest retention time, and T25 the tag with the shortest retention time) are summarized below:

5

T1	10A	T6	10C	T11	7B	T16	5C	T21	2B
T2	9A	T7	9B	T12	7C	T17	4B	T22	2C
T3	8A	T8	9C	T13	6B	T18	4C	T23	1B
T4	7A	T9	8B	T14	6C	T19	3B	T24	1C
T5	10B	T10	8C	T15	5B	T20	3C	T25	2D

10

The designations 10A, 9A, etc. are as described in Example 3.

15 The fifteen reagents used in the first five stages and the code identifying them are represented below where 1 represents the presence of tag and 0 the absence thereof.

-103-

5

10

15

20

REAGENT	CODE
L-serine	(0001)
D-serine	(0010)
L-glutamic acid	(0011)
D-glutamic acid	(0100)
L-glutamine	(0101)
D-glutamine	(0110)
L-lysine	(0111)
D-lysine	(1000)
L-Proline	(1001)
D-Proline	(1010)
L-phenylalanine	(1011)
D-phenylalanine	(1100)
3-amino-benzoic acid	(1101)
4-aminophenyl acetic acid	(1110)
3,5-diamino- benzoic acid	(1111)

-104-

The 31 reagents and the code representing them in the sixth stage are represented below:

REAGENT	CODE
L-serine	(00001)
D-serine	(00010)
L-glutamic acid	(00011)
D-glutamic acid	(00100)
L-glutamine	(00101)
D-glutamine	(00110)
L-lysine	(00111)
D-lysine	(01000)
L-proline	(01011)
D-proline	(01010)
L-phenylalanine	(01011)
D-phenylalanine	(01100)
3-amino-benzoic acid	(01101)
4-aminophenyl acetic acid	(01110)
3,5-diamino-benzoic acid	(01111)
Succinic Anhydride	(10000)
Tiglic acid	(10001)
2-pyrazine carboxylic acid	(10010)

5

10

15

20

-105-

(±)thioctic acid	(10011)
1-piperidinepropionic acid	(10100)
piperonylic acid	(10101)
6-methylnicotinic acid	(10110)
3-(2-thienyl)acrylic acid	(10111)
methyl iodide	(11000)
tosyl chloride	(11001)
p-toluenesulfonyl isocyanate	(11010)
3-cyanobenzoic acid	(11011)
phthallic anhydride	(11100)
acetic anhydride	(11101)
ethyl chloroformate	(11110)
mesylchloride	(11111)

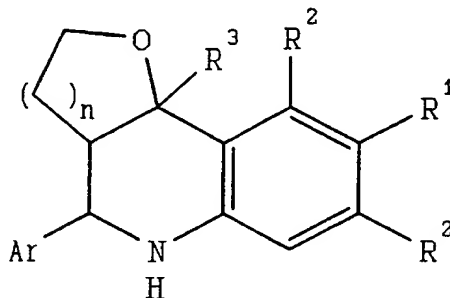
15 A spacer of six glycine units was prepared on the beads  
using standard methods. The variable region was  
constructed using butyl sidechain protection, and amino  
groups were protected as Fmoc derivatives. Amide bonds  
were formed by activation of the carboxylic acid with DIC  
20 and HOBt.

-106-

## EXAMPLE 7

Hetero-Diels-Alder Library

A combinatorial hetero Diels-Alder library comprising 42 compounds of the formula:



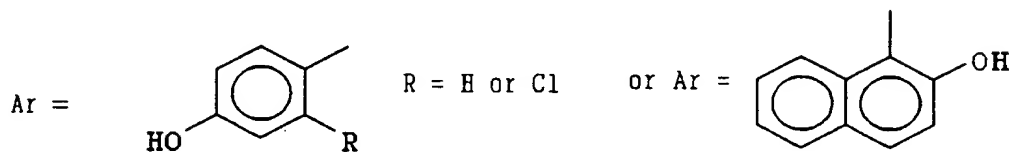
wherein;

$R^1$  is H,  $\text{CH}_3\text{O}$ ,  $\text{F}_3\text{C}$ ,  $\text{F}_3\text{CO}$ ,  $\text{H}_5\text{C}_6\text{O}$ , or  $\text{C}_6\text{H}_{11}$ ;

$R^2$  is H,  $\text{CH}_3$ , or  $\text{CH}_3\text{O}$ ;

$R^3$  is H (when  $n=2$ ), or  $\text{CH}_3$  (when  $n=1$ ); and

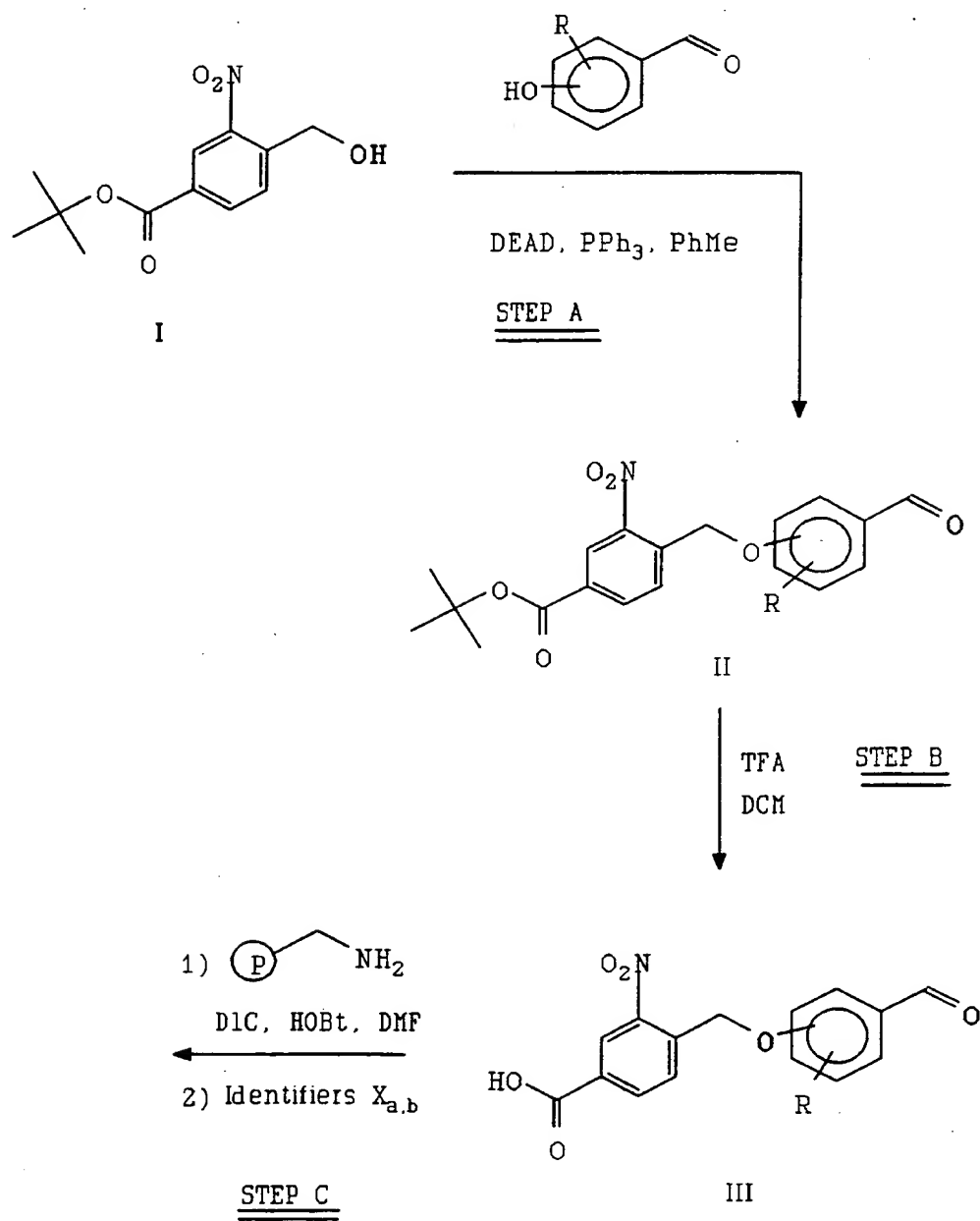
15



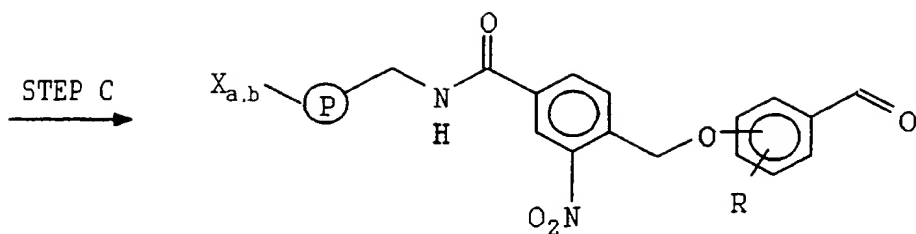


-107-

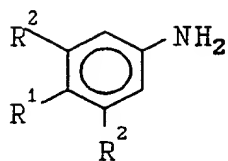
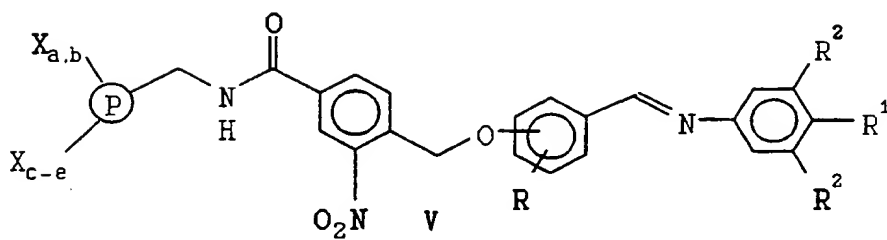
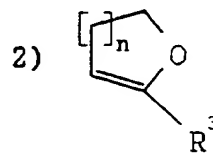
was constructed per the following scheme:



-108-



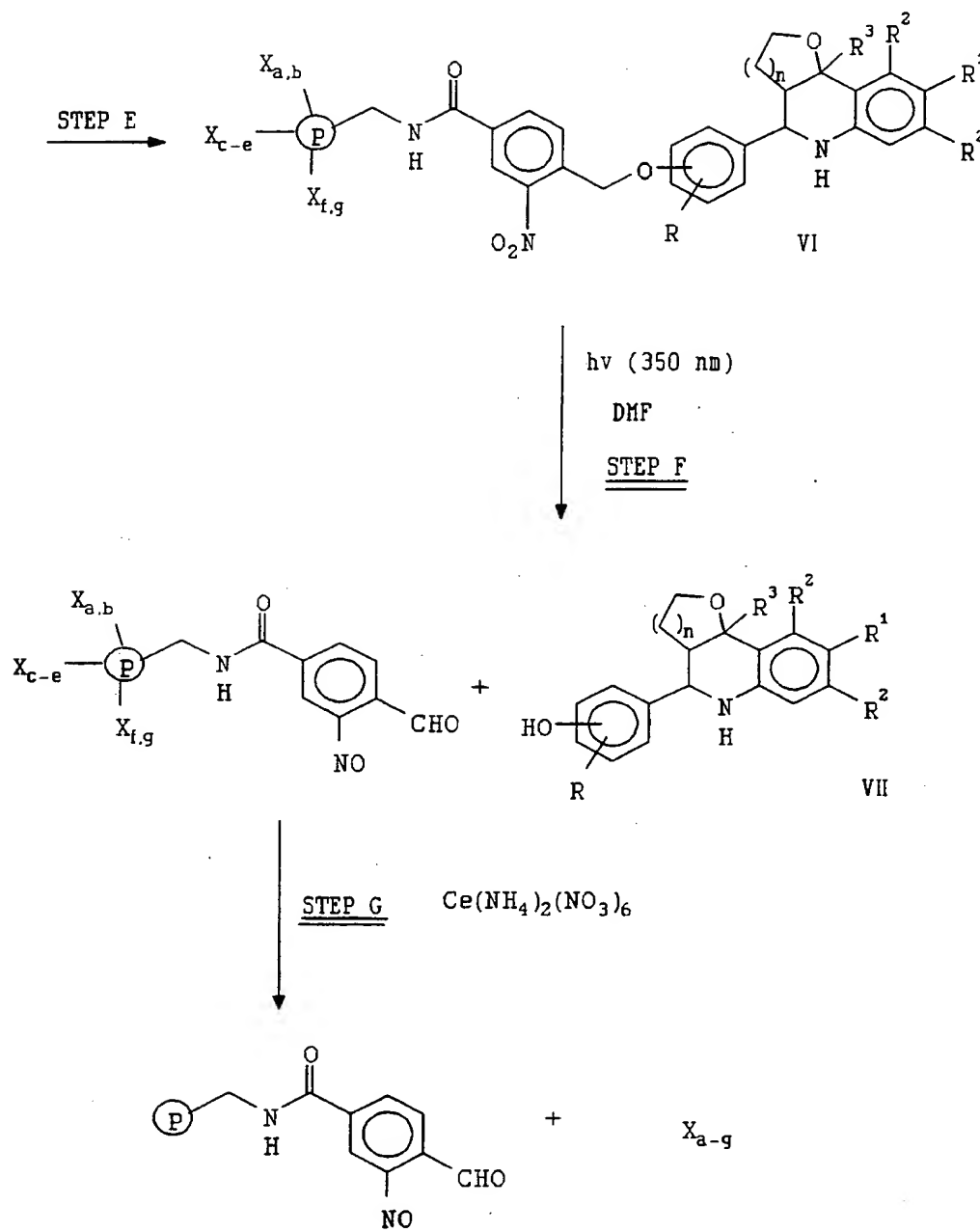
1) Toluene

2) Identifiers  $X_{c-e}$ STEP D1) Identifiers  $X_{t,g}$  $\text{BF}_3 \cdot \text{Et}_2\text{O}$ 

DCM

STEP E

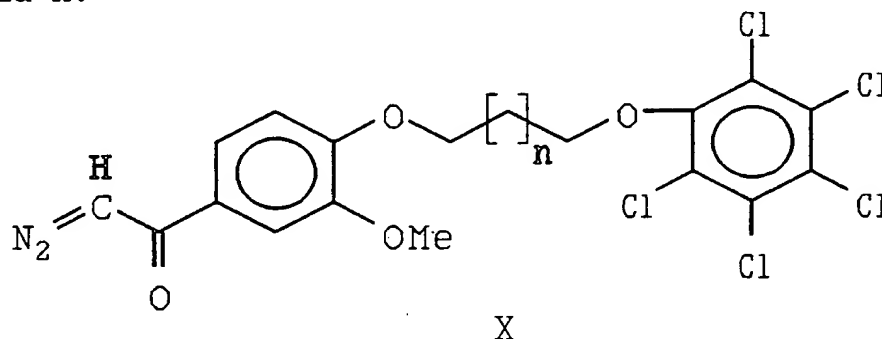
-109-



-110-

The azatricyclic products (VI) were constructed on polystyrene beads and were linked to the beads by a photocleavable linker allowing the azatricycle (VII) to be removed from the bead by exposure to U.V. light (350 nm in DMF). The binary codes introduced in steps C,D and E allow a unique determination of the reaction sequence used to introduce ArR, R<sup>1</sup>, R<sup>2</sup> and R<sup>3</sup>. The encoding tags were removed according to step G and analyzed by electron capture detection following GC separation.

The identifiers used in this scheme are represented by the formula X:



Wherein;

X<sub>a</sub> indicates n=10

X<sub>b</sub> indicates n=9

X<sub>c</sub> indicates n=8

X<sub>d</sub> indicates n=7

X<sub>e</sub> indicates n=6

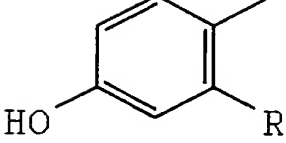
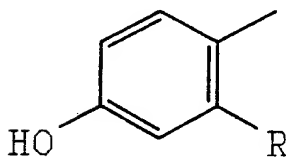
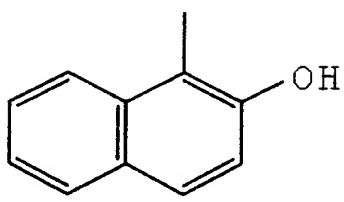
X<sub>f</sub> indicates n=5

X<sub>g</sub> indicates n=4

The codes for each of R, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> are as follows:

-111-

TABLE 7-1

5	<u>X</u>	Ar =		R = H
	a			
10	b	Ar =		R = Cl
15	a, b	Ar =		
<hr/>				
	c		R <sup>1</sup> =H	R <sup>2</sup> =H
20	d		R <sup>1</sup> =H	R <sup>2</sup> =CH <sub>3</sub>
	d, c		R <sup>1</sup> =OCH <sub>3</sub>	R <sup>2</sup> =OCH <sub>3</sub>
	e		R <sup>1</sup> =CF <sub>3</sub>	R <sup>2</sup> =H
25	e, c		R <sup>1</sup> =C <sub>6</sub> H <sub>5</sub> O	R <sup>2</sup> =H
	e, d		R <sup>1</sup> =F <sub>3</sub> CO	R <sup>2</sup> =H

-112-

e, d, c                       $R^1 = C_6H_{11}$        $R^2 = H$

f                               $R^3 = CH_3$        $n = 1$

5      g                               $R^3 = H$        $n = 2$

#### Step A

To a solution of I (2.03 g, 8 mmol), 4-hydroxybenzaldehyde (1.17 g, 9.6 mmol) and triphenylphosphine (2.73 g, 10.4 mmol) in toluene (20 mL) stirring at 0°C was added over a period of 30 minutes diethylazodicarboxylate. The solution was allowed to warm and stirred for 1 hour once ambient temperature had been reached. The solution was concentrated by removal of approximately half of the solvent in vacuo and was then triturated with ether. The mixture was then filtered and the residue was washed thoroughly with ether. The solvent was removed in vacuo and the residue was purified by chromatography on silica gel (15% ethyl acetate in hexane) affording 1.3 g of the ether IIa (47% yield).

2-chloro-4-hydroxybenzaldehyde and 2-hydroxy-1-naphthaldehyde were coupled to I in analogous fashion affording ethers IIb and c in yields of 91% and 67%, respectively.

#### Step B

To a solution of ether IIa (0.407 g, 1.14 mmol) in DCM (20 mL) stirring at room temperature was added TFA (8 mL).

-113-

The solution was allowed to stir for 6 hrs. The solution was evaporated to dryness in vacuo affording 0.343 g of acid IIIa (100% yield). Ethers IIb and IIc were deprotected analogously affording acids IIb and c in yields of 92% and 100% respectively.

### Step C

Into a peptide reaction vessel (Merrifield vessel) were measured 1% DVB (divinylbenzene) cross-linked polystyrene beads (50-80 $\mu$ ) functionalized with aminomethyl groups (1.1 meq/g) (200 mg of resin). The resin was suspended in DMF (2 mL) and shaken for 20 min. The acid IIIa (38 mg, 2 equiv.), 1-hydroxybenzotriazole (40 mg, 2 equiv) and diisopropylcarbodiimide (38 mg, 2 equiv) were added and the mixture shaken until a negative Ninhydrin test was achieved (22 hr). The solution was removed by filtration and the resin was washed with DCM (8x 10 mL).

The resin was resuspended in DCM (5 mL), identifier Xa (15 mg) was added and the flask was shaken for 1 hr. Rh(TFA)<sub>2</sub> catalyst (1 mol%) was added and the flasks shaken for 2 hrs. The solvent was removed by filtration and the resin resuspended in DCM (5 mL). Trifluoroacetic acid (1 drop) was added and the vessel shaken for 20 min. The solvent was removed by filtration, and the resin was washed with DCM (8x 10 mL).

-114-

In an analogous fashion, acids IIIb and IIIc were attached to the resin and were encoded with the appropriate identifiers, i.e., Xb for acid IIIb and Xa and Xb for acid IIIc. The three batches of resin were combined, mixed, washed, and dried.

#### Step D

The dry resin was divided into 7 equal portions (87 mg) which were put into seven peptide reaction vessels (Merrifield vessels) which were wrapped with heat tape. The resin in each vessel was suspended in toluene (10 mL) and shaken for 20 min. An appropriate amount of one aniline was then added to each flask (see Table 7-2).

TABLE 7-2

FLASK	ANILINE	AMOUNT ADDED
1	Aniline	3 mL
2	3,5-dimethylaniline	3 mL
3	3,4,5-trimethoxyaniline	2 g
4	4-trifluoromethylaniline	3 mL
5	4-phenoxyaniline	2 g
6	4-trifluoromethoxyaniline	3 mL
7	4-cyclohexylaniline	2 g

The heating tape was connected and the reaction mixtures shaken at 70°C for 18 hrs. The heat tape was disconnected and the solvent was removed by filtration and each batch of resin was washed with dry DCM (4x 10 mL), ether (10 mL), toluene (10 mL) and DCM (2x 10 mL). Each of the portions was then suspended in DCM (5 mL) and to each flask was added the appropriate identifier or



-115-

combination of identifiers (Xc-e) (15 mg) (see Table 7-1). The flasks were shaken for 1 hr. and then Rh(TFA)<sub>2</sub> (1 mol%) was added to each flask and shaking continued for 2 hrs.

5 The solvent was then removed and each batch of resin was re-suspended in DCM (5 mL) to which was added TFA (1 drop). This mixture was shaken for 20 min., then the solvent was removed by filtration. The batches of resin were then washed (DCM, 1x 10 mL) and combined, washed  
10 again with DCM (3x 10 mL) and then dried thoroughly in vacuo.

#### Step E

The dried resin was divided into two equal portions (0.3  
15 g) and each was placed in a peptide reaction vessel. The resin batches were washed with DCM (2x 10 mL) and then resuspended in DCM (5 mL). To one flask was added the identifier Xf (15 mg) and to the other was added Xg (15 mg). The flasks were shaken for 1 hr. prior to the  
20 addition of Rh(TFA)<sub>2</sub> catalyst (1 mol%). The flasks were shaken for 2 hrs. and then the solvent was removed by filtration. Each batch of resin was washed with DCM (3x 10 mL), and each was then resuspended in DCM (5 mL).

25 The appropriate enol ether (1 mL) (see Table 7-1) was added to the flasks and the vessels shaken for 30 min. To each flask was added a solution of BF<sub>3</sub>•OEt<sub>2</sub> (0.5 mL of a 5% solution in DCM) and the flasks were shaken for 24 hrs. Removal of the solvent by filtration was followed by  
30 washing of the resin with DCM (10 mL) and the resin was then combined. The beads were then washed further with DCM (5x 10 mL), DMF (2x 10 mL) methanol (2x 10 mL) and DCM (2x 10 mL). The resin was then dried thoroughly in vacuo.

#### 35 Step F

To confirm the identity of the products produced in the Hetero-Diels-Alder library one example was completed on a

-116-

large scale to allow confirmation of the structure by spectroscopic means. The procedure followed was essentially the same method as described for the combinatorial library. In step A 4-hydroxybenzaldehyde was coupled to the photolabile group. In step D, aniline was condensed with the aldehyde. In step E, the enol ether was formed with 4,5-dihydro-2-methylfuran.

The photolysis of the compound (step F) was performed by suspending 100 mg of the beads in DMF (0.3 mL) and irradiating the beads with UVP "Black Ray" model UVL 56 hand-held 366 nm lamp for 16 hrs. The DMF was removed to one side by pipette and the beads rinsed with additional DMF (2x 3 mL). The original solution and the washings were combined and the solvent removed in vacuo. NMR analysis of the reaction mixture showed it to contain the desired azatricycle by comparison to the authentic sample.

#### Step G

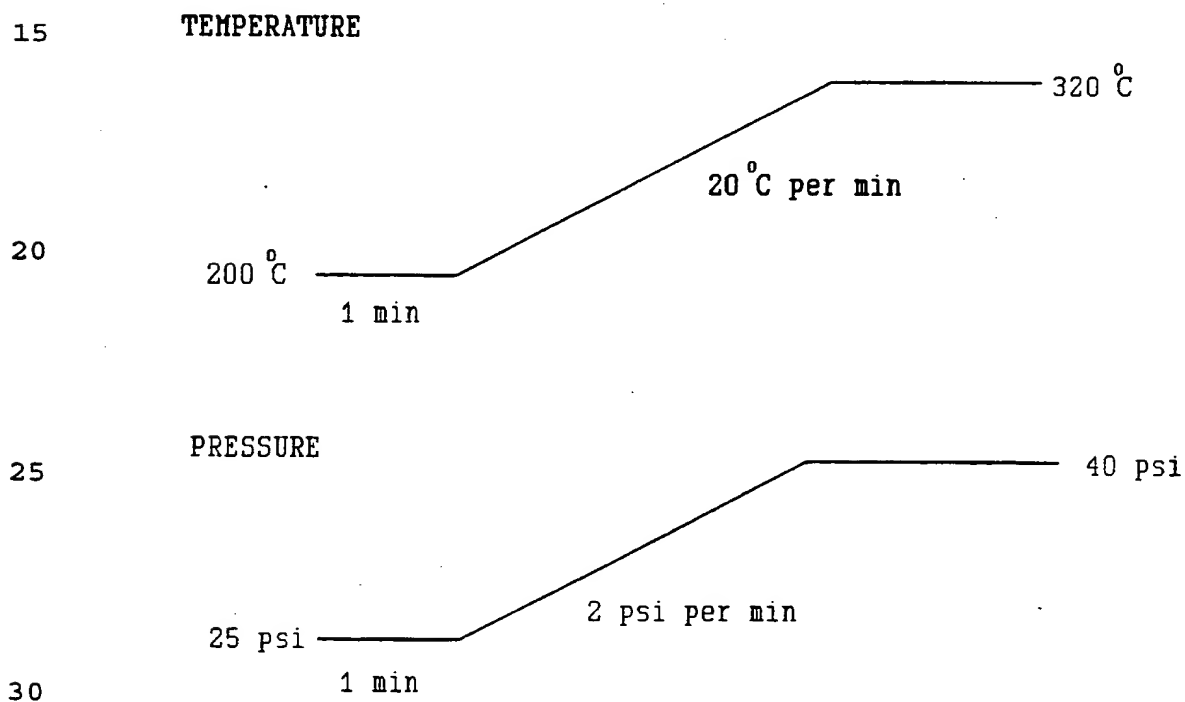
A bead of interest was placed into a pyrex glass capillary tube sealed at one end. A solution (1  $\mu$ L) of 1M aqueous cerium (IV) ammonium nitrate and acetonitrile (1:1) was syringed into the tube, and the tube was then centrifuged so that the bead lay on the bottom of the capillary and was completely immersed by the reagent solution. Hexane (2  $\mu$ L) was added by syringe and the tube was again centrifuged. The open end of the capillary was flame-sealed and placed in an ultrasonic bath for 4 hrs. The capillary was then placed inverted into a centrifuge and spun such that the aqueous layer was forced through the hexane layer to the bottom of the tube. This extraction process was repeated 3 or 4 times and the tube was then opened. The hexane layer (1.5  $\mu$ L) was removed by syringe and placed into a different capillary containing BSA (0.2  $\mu$ L). This tube was sealed and centrifuged until the reagents were thoroughly mixed. A portion of the solution (ca. 1  $\mu$ L) was removed and injected into a gas

-117-

chromatography machine with a 25M x 0.2 mM methylsilicone fused silica column with electron capture detection for separation and interpretation of the tag molecules.

- 5 The sample was injected onto the GC column at 200°C and 25  
psi of carrier gas (He<sub>2</sub>). After 1 minute the temperature  
was increased at a rate of 20°C per minute to 320°C, and  
the pressure was increased at a rate of 2 psi per minute  
to 40 psi. These conditions are shown in the following  
10 diagram:

## GC CONDITIONS



-118-

The following results were obtained with four randomly selected beads:

Bead 1

5

	TAG DETECTED				
	Xf	Xe	Xd	Xc	Xb Xa
Ar					2-Hydroxy naphthyl
R <sup>1</sup>		C <sub>6</sub> H <sub>11</sub>			
R <sup>2</sup>		H			
R <sup>3</sup>	CH <sub>3</sub> (n=1)				

10

Bead 2

15

TAG DETECTED					
	Xg	Xe	Xd	Xc	Xb
Ar					2-chloro-4-hydroxyphenyl
R <sup>1</sup>		C <sub>6</sub> H <sub>11</sub>			
R <sup>2</sup>		H			
R <sup>3</sup>	H (n=2)				

Bead 3

20

25

	TAG DETECTED		
	Xg	Xe Xd	Xb Xa
Ar			2-Hydroxy naphthyl
R <sup>1</sup>		F <sub>3</sub> CO	
R <sup>2</sup>		H	
R <sup>3</sup>	H (n=2)		

Bead 4

30

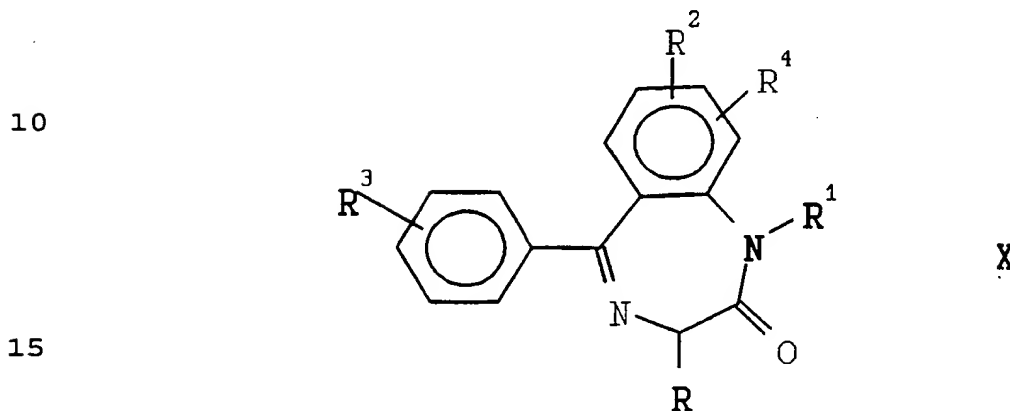
	TAG DETECTED			
	Xf	Xe	Xd	Xb
Ar				2-chloro-4-hydroxyphenyl
R <sup>1</sup>		F <sub>3</sub> CO		
R <sup>2</sup>		H		
R <sup>3</sup>	CH <sub>3</sub> (n=1)			

-119-

## EXAMPLE 8

Benzodiazepine Library

Following the procedure of Example 4, a combinatorial  
5 library is constructed of the Formula X



wherein

20 R is a radical of a naturally occurring D or L amino acid;  
R<sup>1</sup> is H, C<sub>1</sub>-C<sub>6</sub> alkyl, lower alkenyl, C<sub>1</sub>-C<sub>6</sub> alkylamine,  
carboxy C<sub>1</sub>-C<sub>6</sub> alkyl, or phenyl C<sub>1</sub>-C<sub>6</sub> alkyl wherein the  
phenyl is optionally substituted by lower alkyl, F, Cl,  
Br, OH, NH<sub>2</sub>, CO<sub>2</sub>H, or O-lower alkyl;

25 R<sup>2</sup> is H or CO<sub>2</sub>H;

R<sup>3</sup> is H or OH;

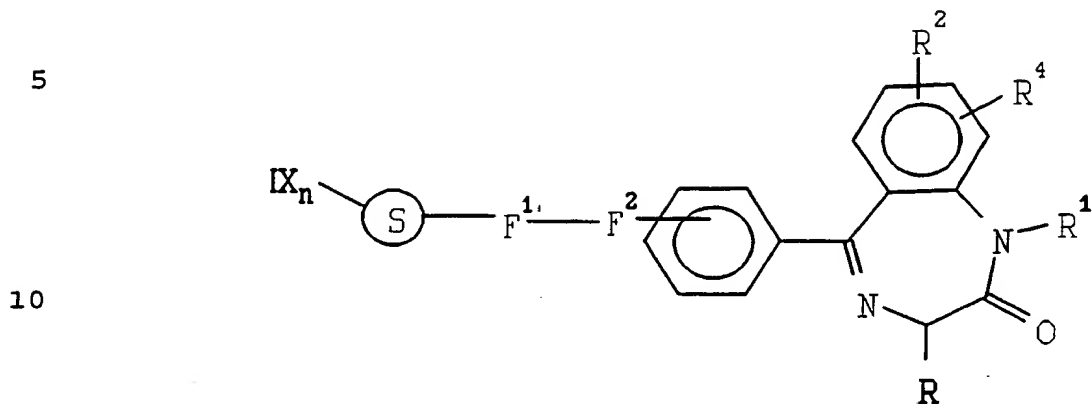
R<sup>4</sup> is H or Cl;

with the provisos that when R<sup>3</sup> is OH, R<sup>2</sup> is H and when R<sup>2</sup>  
is carboxy, R<sup>3</sup> is H.

30

-120-

This library is released from a plurality of encoded beads of the general formula



15 wherein  
 IX<sub>n</sub> is a plurality of identifiers of the Formula Ia wherein  
 said plurality represents an encoded scheme;  
 S is a substrate;  
 F<sup>1</sup>'-F<sup>2</sup> is the residue of the linker member of Formula Ia;  
 20 and  
 R, R<sup>1</sup>, R<sup>2</sup>, and R<sup>4</sup> are as defined for Formula X.

-121-

## EXAMPLE 9

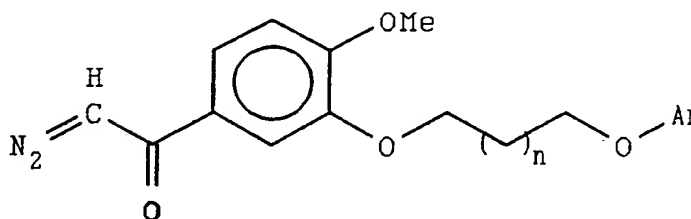
Typical Identifier Preparations

The diazo compound identifiers which are attached to the resin via carbene formation are prepared as exemplified.

5

Compounds of the general formula

10



15

wherein

$n$  is 0-10 and

Ar is pentachlorophenol, 2,4,6-trichlorophenol,

20 2,4,5-trichlorophenol, or 2,6-dichloro-4-fluorophenol  
are prepared as follows.

To a solution of 1-hydroxy-4-(2,6-dichloro-4-fluorophenoxy)butane (0.38 g, 1.5 mmol), isovanillin (0.228 g, 1.5 mmol) and triphenylphosphine (0.393 g, 1.5 mmol) in THF (8 mL) was added diethylazodicarboxylate (0.287 g, 1.7 mmol). The solution stirred at r.t. for 36 hrs. The solvent was removed in vacuo and the residue purified by chromatography on silica gel (with a mixture of 20% ethyl acetate and 80% petroleum ether) affording 0.45 g of the aldehyde (77% yield).

The aldehyde (100 mg, 0.26 mmol) was dissolved in acetone (8 mL) and was treated with a solution of  $\text{KMnO}_4$  (61 mg, 0.39 mmol) in acetone (4 mL) and water (4 mL). The reaction stirred at room temperature for 13 hrs. The mixture was diluted with ethyl acetate (100 mL) and water

35

-122-

(50 mL) and the layers were separated. The aqueous layer was extracted with additional ethyl acetate (2x 100 mL). The combined organic layers were washed with water (50 mL) and dried ( $\text{MgSO}_4$ ). Removal of the solvent afforded 109 mg of the benzoic acid (93% yield).

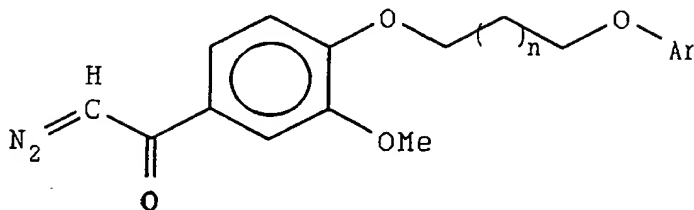
A solution of the acid (76 mg, 0.188 mmol) in methylene chloride (2 mL) was treated with oxalylchloride (36 mg, 0.28 mmol) and catalytic DMF. After stirring for 10 min at room temperature slow but steady evolution of gas was observed. Stirring continued for 2 hrs. when the solution was diluted with DCM (15 mL) and washed with saturated aqueous sodium hydrogencarbonate solution (5 mL). The layers were separated. The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ) and the solvent evaporated affording the benzoyl chloride as pale yellow crystals.

The benzoyl chloride was dissolved in methylene chloride (5 mL) and was added to a stirring solution of an excess of diazomethane in ether at  $-78^\circ\text{C}$ . The cold bath was allowed to warm up and the mixture allowed to stir for 5 hrs at room temperature. The solvents and excess diazomethane were removed in vacuo and the residue purified by chromatography on silica gel using gradient elution method where the concentration of ethyl acetate ranged from 10% to 40 % in hexanes affording 48 mg of the diazo compound (60% yield).



-123-

Compounds of the general formula:



wherein;

15 n is 0-10 and

Ar is pentachlorophenol, 2,4,6-trichlorophenol, 2,4,5-trichlorophenol, or 2,6-dichloro-4-fluorophenol are prepared as follows.

20 Methyl vanillate (0.729 g, 4.0 mmole), 1-hydroxy-9-(2,3,4,5,6-pentachlorophenoxy)nonane (1.634 g, 4.0 mmole) and triphenylphosphine (1.259 g, 4.8 mmole) were dissolved in 20 mL dry toluene under argon. DEAD (0.76 mL, 0.836 g, 4.8 mmole) was added dropwise, and the mixture was stirred  
25 at 25°C for one hour. The solution was concentrated to half volume and purified by flash chromatography eluting with DCM to give 1.0 g (1.7 mmole, 43%) of the product as a white crystalline solid.

30 The methyl ester above (1.0 g, 1.7 mmole) was dissolved in 50 mL THF, 2 mL water was added followed by lithium hydroxide (1.2 g, 50 mmole). The mixture was stirred at 25°C for one hour then refluxed for five hours. After cooling to 25°C the mixture was poured onto ethyl acetate  
35 (200 mL) and the solution was washed with 1 M HCl (50 mL x3) then sat. aq. NaCl (1x 50 mL) and dried over sodium

-124-

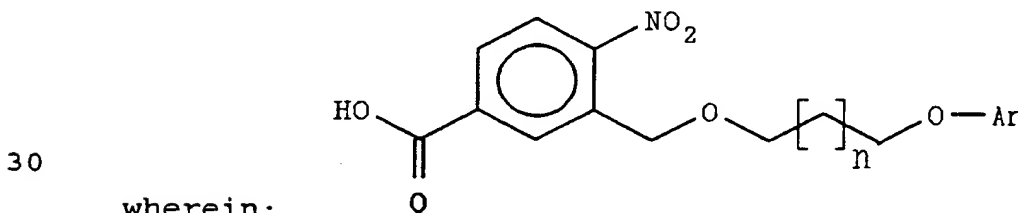
sulfate. The solvent was removed and the crude acid azeotroped once with toluene.

The crude material above was dissolved in 100 mL toluene, 10 mL (1.63 g, 14 mmole) thionyl chloride was added, and the mixture was refluxed for 90 min. The volume of the solution was reduced to approximately 30 mL by distillation, then the remaining toluene removed by evaporation in vacuo. The crude acid chloride was dissolved in 20 mL dry DCM and cooled to -78°C under argon and a solution of approximately 10 mmole diazomethane in 50 mL anhydrous ether was added. The mixture was warmed to room temperature and stirred for 90 min. Argon was bubbled through the solution for 10 min. then the solvents were removed by evaporation in vacuo and the crude material was purified by flash chromatography eluting with 10-20% ethyl acetate in hexane. The diazoketone (0.85 g, 1.4 mmole, 82% over three steps) was obtained as a pale yellow solid.

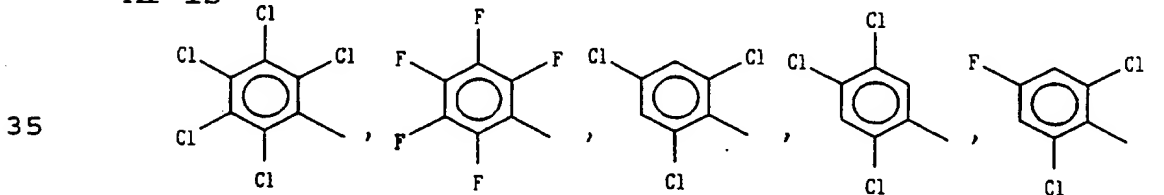
The following identifiers have been prepared as described above:

#### Photolabile Cleavage

50 Identifiers were prepared of the formula:



Ar is

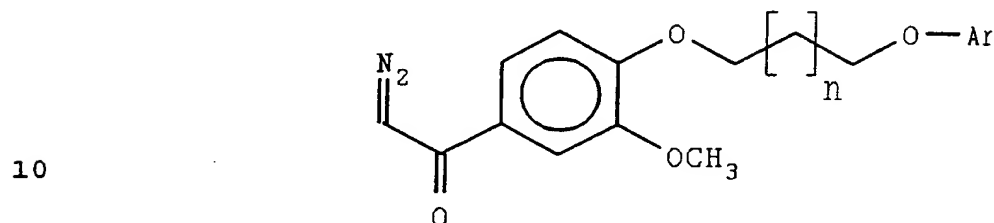


-125-

and n is 1,2,3,4,5,6,7,8,9, and 10.

**Oxidative Cleavage Type I**

5 7 Identifiers were prepared of the formula



wherein:

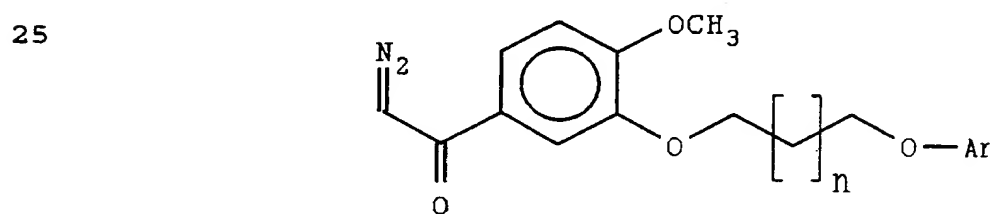
Ar is



20 and n is 4,5,6,7,8,9, and 10.

**Oxidative Cleavage Type II**

13 Identifiers were prepared of the formula



30 wherein:

Ar is



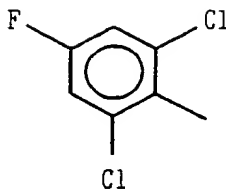
-126-

and n is 1,2,3,4,5,6,7,8,9,10;

and wherein:

3Ar is

5



10 and n is 0,3, and 9.

15

-127-

## Example 10

Encoding Combinatorial Libraries with Tags Readable by Mass Spectroscopy

5 The tags 4, 11 and 13 (Scheme 8) of the same structure, but different molecular weights due to varying deuterium substitution, were each synthesized (Schemes 9 and 10) and separately analyzed by mass spectroscopy (MS). Among MS techniques, positive chemical ionization mass spectroscopy (PCIMS) gave minimal fragmentation of the tag, such that  
10 only the molecular ion ( $[M+NH_4]^+$ ) and one other fragment ( $[MH-H_2O]^+$ ) were evident (Figures 1, 2 and 3). This actually allowed the presence or absence of a tag to be determined by the observation of two signals, which removes any possible ambiguity when analyzing a more  
15 complex mixture. Approximately equal amounts of the three tags were then mixed and analyzed by PCIMS (figure 5). Again, the two signals corresponding to each separate tag could easily be distinguished.

20 Tag 4 was now transformed into the diazoketone precursor 8 (Scheme 9), then attached to Tentagel resin as 9 (Scheme 12). One bead of the 9 was subsequently removed and 4 oxidatively released using ceric ammonium nitrate. PCIMS analysis again clearly showed the presence of tag 4.

25 In summary, the set of tags 4, 11 and 13 of the same structure, but different molecular weights were synthesized. All were easily detected simultaneously in a mixture by PCIMS. The small amount of 4 released from  
30 a single bead of Tentagel resin used in combinatorial library synthesis is detectable by PCIMS. MS is a viable and sensitive detection method for tags, and can be used as the basis for an encoding scheme of a combinatorial library.

35 Analysis of 4, 11 and 13 by PCIMS was obtained using a reagent gas mixture of 1%  $NH_3$  in  $CH_4$ .

-128-

(2). To a solution of 11.1 mL (125 mmole, 5.00 eq.) of 1,4-butanediol (1), 6.97 mL (50.0 mmole, 2.00 eq.) of Et<sub>3</sub>N and 0.153 g (1.25 mmole, 0.05 eq.) of 4-dimethylaminopyridine in dry CH<sub>2</sub>Cl<sub>2</sub> (100 mL) at 0°C under Ar, was added 3.88g (25.0 mmole, 1.00 eq.) of 97% tert-butyldimethylsilyl chloride. The resulting solution was stirred at 0°C for 15 min, then 25°C for 16 hours. The reaction was then diluted with CH<sub>2</sub>Cl<sub>2</sub> (250 mL) and washed with 1 M HCl (100mL), saturated aq. NaHCO<sub>3</sub> (100 mL) and H<sub>2</sub>O (100 mL), then dried (MgSO<sub>4</sub>). Removal of the volatiles in vacuo gave the crude product 2 as an oil.

(3). To a solution of ~10.0 mmole of crude alcohol 2, 1.93 g (10.5 mmole, 1.05 eq.) of pentafluorophenol and 2.89 g (11.0 mmole, 1.10 eq.) of triphenylphosphine in dry CH<sub>2</sub>Cl<sub>2</sub> (40 mL) at 0°C under Ar, was added 1.73 mL (11.0 mmole, 1.10 eq.) of diethyl azodicarboxylate. The resulting orange solution was stirred at 0° C for 5 min, then 25°C for 15 hours. The reaction was then diluted with CH<sub>2</sub>Cl<sub>2</sub> (250 mL) and washed with saturated aq. Na<sub>2</sub>CO<sub>3</sub> (100 mL), saturated aq. NH<sub>4</sub>Cl (100mL) and H<sub>2</sub>O (100 mL), then dried (MgSO<sub>4</sub>). Removal of the volatiles in vacuo and purification by flash chromatography (0-20% EtOAc in hexanes) gave the product 3 as an oil.

(4). To a solution of 1.85 g (5.00 mmole, 1.00 eq.) of silyl-protected alcohol 3 in THF (20 mL) at 25°C, was added 10.0 ML (10.0 mmole, 2.00 eq.) of a 1.0 M solution of tetrabutylammonium fluoride in THF. The resulting orange solution was stirred at 25°C for 4 hours. Removal of the volatiles in vacuo and purification by flash chromatography (20-40% EtOAc in hexanes) gave 1.10 g (86%) of the product 4 as an oil.

(5). To a solution of 0.800 g (3.125 mmole, 1.00 eq.) of alcohol 4, 0.569 g (3.125 mmole, 1.00 eq.) of methyl vanillate and 0.984 g (3.75 mmole, 1.20 eq.) of

-129-

triphenylphosphine in dry  $\text{CH}_2\text{Cl}_2$  (20 mL) at  $0^\circ\text{C}$  under Ar, was added 0.591 mL (3.75 mmole, 1.20 eq.) of diethyl azodicarboxylate. The resulting pale yellow solution was stirred at  $0^\circ\text{C}$  for 5 min, then  $25^\circ\text{C}$  for 19 hours. The reaction was then diluted with  $\text{CH}_2\text{Cl}_2$  (100 mL) and washed with 1 M NaOH (50 mL), saturated aq.  $\text{NH}_4\text{Cl}$  (50 mL) and  $\text{H}_2\text{O}$  (50 mL), then dried ( $\text{MgSO}_4$ ). Removal of the volatiles in vacuo and purification by flash chromatography (20% EtOAc in hexanes) gave the product 5 as an oil.

(6). To a solution of 3.125 mmole of ester 5 in THF (12 mL) was added 1.31 g (31.3 mmole, 10.0 eq.) of lithium hydroxide monohydrate. MeOH (24 mL) was added to the resulting suspension to form a solution, which was stirred at  $25^\circ\text{C}$  for 1 hours, then refluxed for 1 day. Volatiles were removed in vacuo, and 1 M HCl then added until solution was ~pH 1. The white precipitate of product which formed was collected and dried to give 0.968 g (76% - 2 steps) of 6.

(7). To 0.968 g (2.38 mmole, 1.00 eq.) of carboxylic acid 6 under Ar, was added 2.43 mL of thionyl chloride. The resulting suspension was refluxed for 1.5 hours, after which time a yellow solution had formed. Volatiles were removed in vacuo, and the resulting residue azeotroped three times with toluene to give the product 7 as colorless crystals.

(8). To a solution of 2.38 mmole of acid chloride 7 in 1:1 THF:MeCN (20 mL) at  $0^\circ\text{C}$  under Ar, was added 1.16 mL (8.33 mmole, 3.50 eq.) of  $\text{Et}_3\text{N}$  followed by 3.57 mL (7.14 mmole, 3.00 eq.) of a 2.0 M solution of (trimethylsilyl)diazomethane in hexanes. The resulting yellow solution was stirred at  $0^\circ\text{C}$  for 1h, then  $25^\circ\text{C}$  for 1 day. The reaction was diluted with EtOAc (150 mL) and washed with saturated aq.  $\text{NaHCO}_3$  (2 x 75 mL) and saturated

-130-

aq. NaCl (2 x 75 mL), then dried (MgSO<sub>4</sub>). Removal of the volatiles *in vacuo* gave the crude product 8 as an oil.

5 (11). Using commercially available 1,4-butanediol-2,2,3,3-d<sub>4</sub> (10) in place of 1, and an analogous procedure to that described for the transformation 1 into 4, 11 was obtained in 41% yield over three steps.

10 Using commercially available 1,4-butanediol-2,2,3,3,4,4-d<sub>6</sub> (10) in place of 1, and an analogous procedure to that described for the transformation 1 into 4, 13 was obtained in 42% yield over three steps.

15 Tag 4 was introduced onto the solid support using 5 to 50% (w/w vs. resin) precursor diazoketone 8 to give 9 by essentially the same procedure given in Example 4; the Hetero Diels-Alder library. Tag 4 was also subsequently removed from 9 by essentially the same procedure as Step G in Example 4.

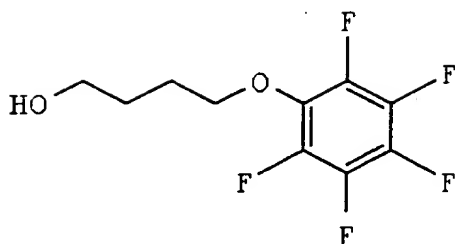
20

The diazoketones corresponding to tags 11 and 13 are used to introduce these tags onto the solid support, so that along with 8, they yield members of a binary encoding set.

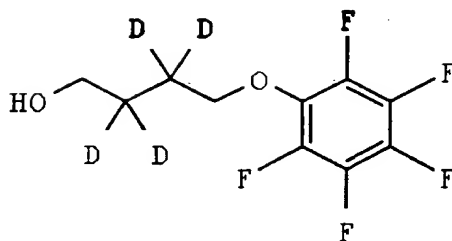


-131-

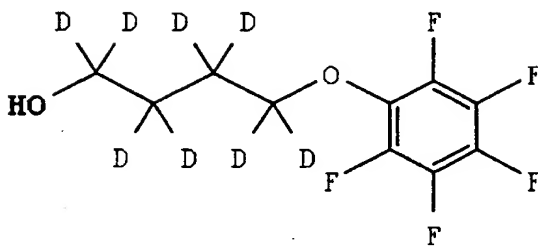
## Scheme 8

**4**

M.W. = 256

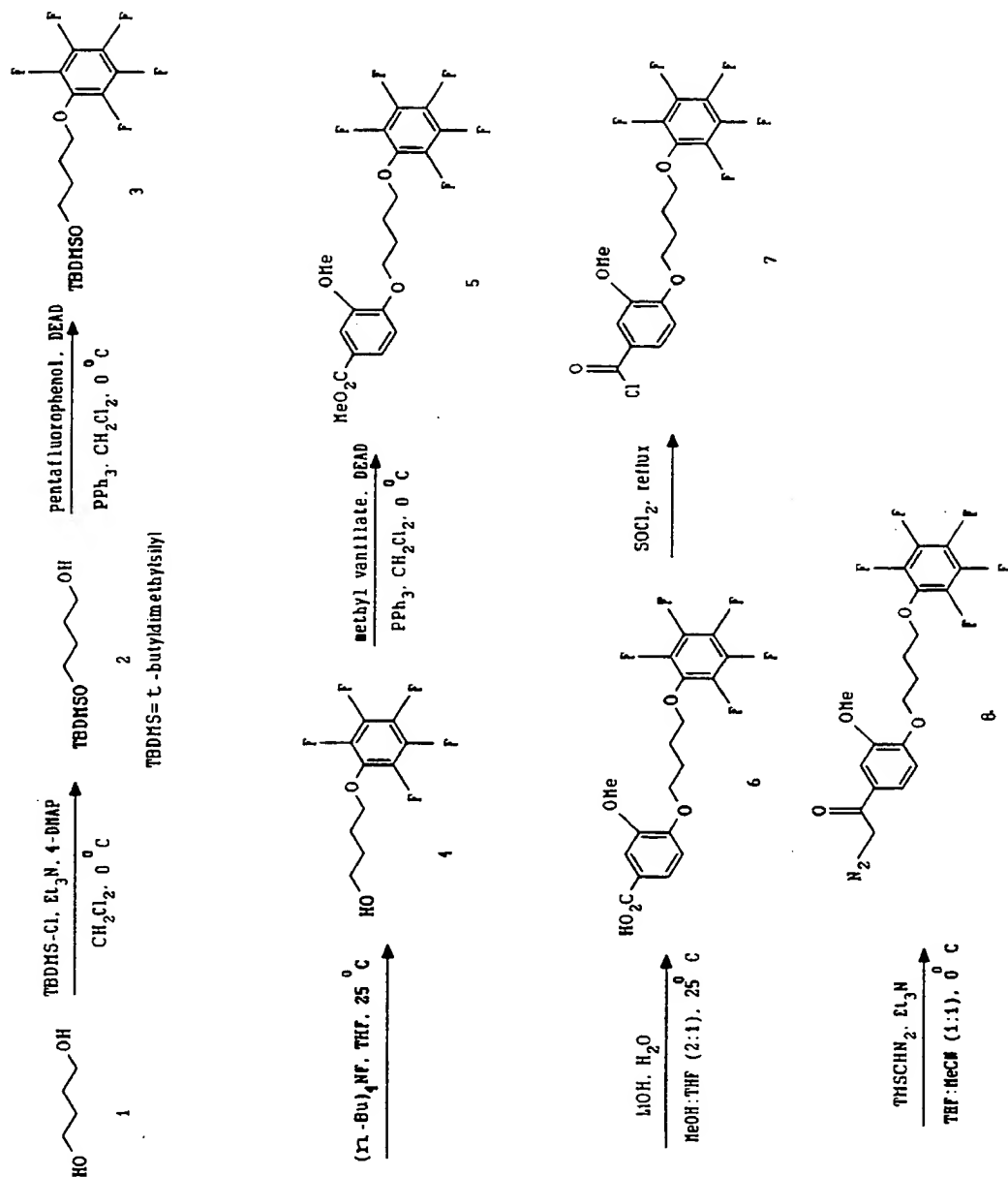
**11**

M.W. = 260

**13**

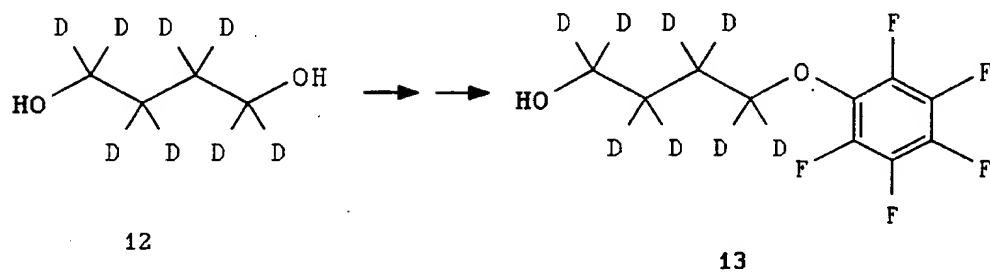
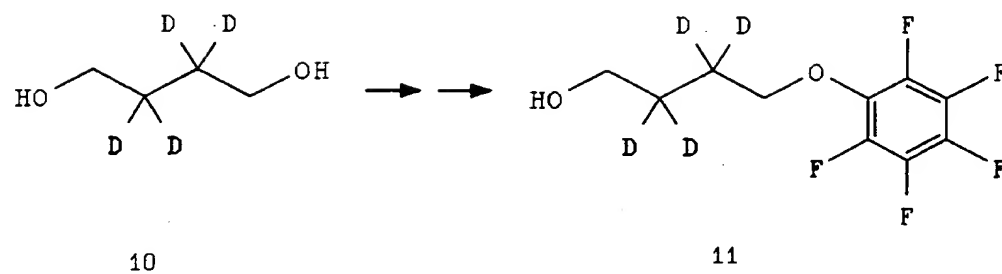
M.W. = 264

## Scheme 9

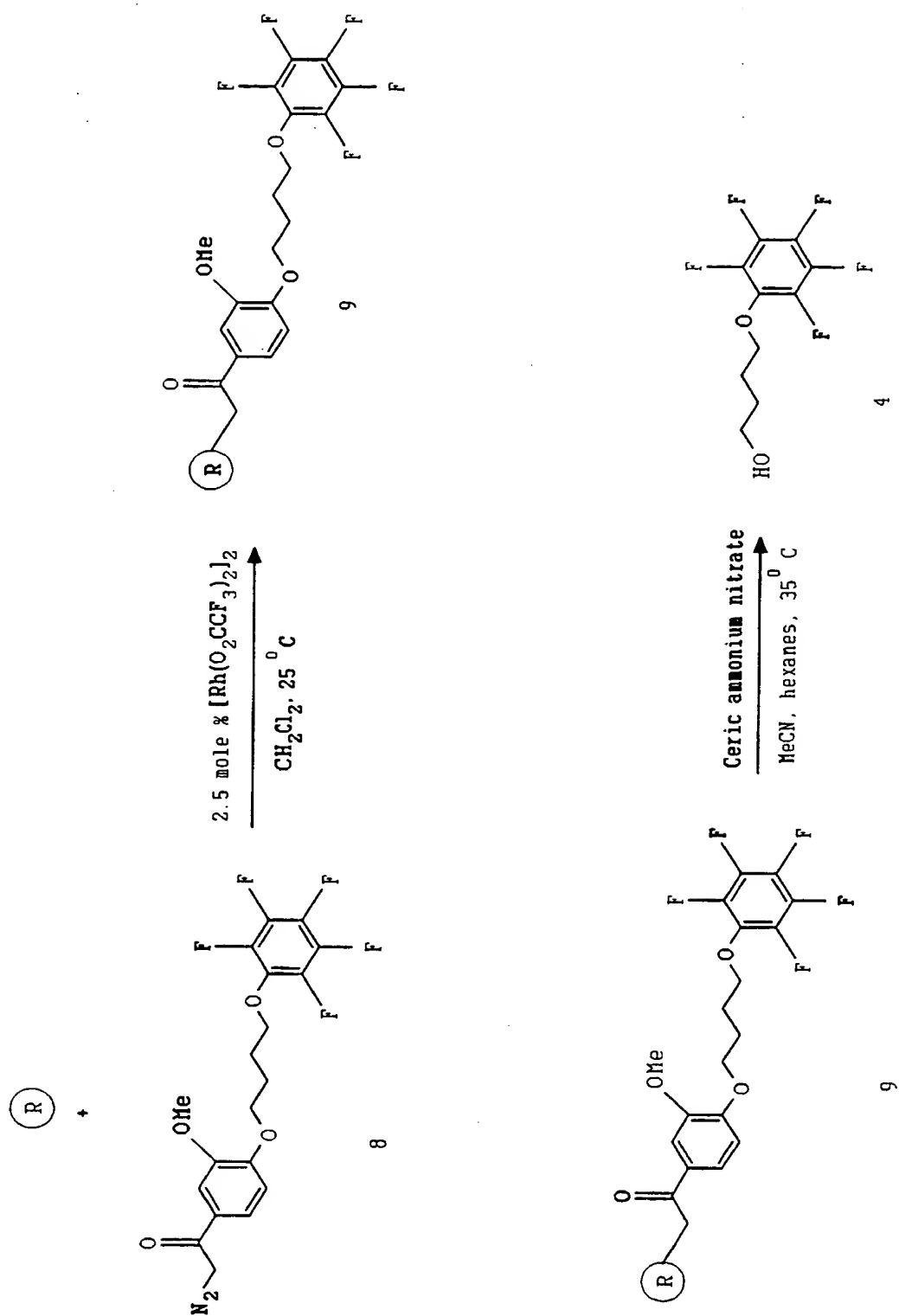


-133-

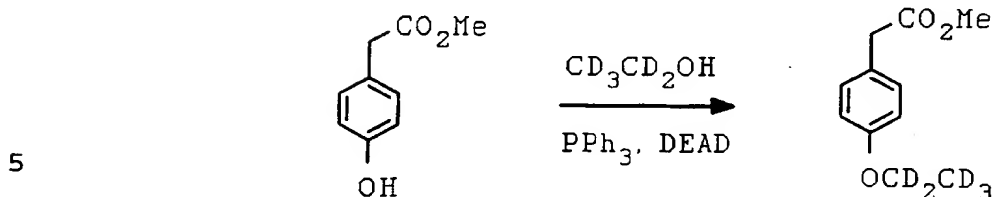
## Scheme 10



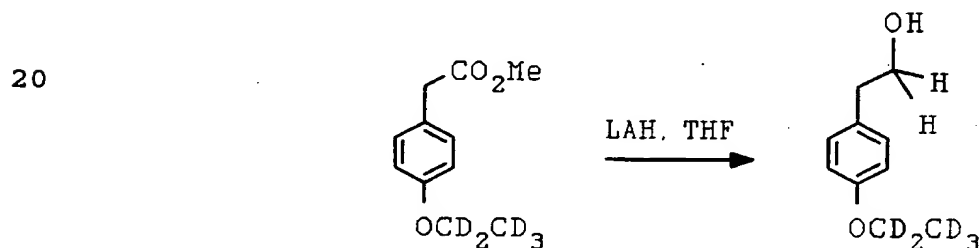
Scheme 11



-135-

Preparation of Deuteriated Tags

10 To a solution of  $\text{PPh}_3$  (3.2 g, 12.1 mmol) in THF (30 mL) at 0 °C was added diethyl azodicarboxylate (1.9 ml, 12 mmol). After 10 minutes at 0 °C, a solution of methyl 4-hydroxyphenylacetate (1.66 g, 10 mmol) and  $\text{d}_5$ -ethanol (800  $\mu\text{L}$ , 12.3 mmol) in 10 mL THF was added. After stirring at 25 °C for 2 hours, the reaction mixture was concentrated and purified by flash chromatography to give methyl 4- $\text{d}_5$ ethoxyphenylacetate (1.86 g, 93%) as a colorless oil.



25 To a solution of 4- $\text{d}_5$  ethoxyphenylacetate (1 g, 5 mmol) in dry ethyl ether at 0 °C was added lithium aluminum hydride (380 mg, 10 mmol). After stirring at 25 °C for 8 hours, the reaction mixture was poured into cold 3 M HCl. The aqueous solution was then extracted with ethyl ether twice. The organic layers were combined, washed with brine and dried over  $\text{MgSO}_4$ . Removal of the solvent gave 4- $\text{d}_5$  ethoxyphenethyl alcohol (850 mg, 100%) as a white solid.

35

-136-

$^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 2.79 (2H, t,  $J = 6.6$  Hz), 3.80 (2H, t,  $J = 6.5$  Hz), 6.83 (2H, d,  $J = 8.6$  Hz), 7.12 (2H, d,  $J = 8.6$  Hz).

5 To a solution of 4- $\text{d}_5$  ethoxyphenylacetate (800 mg, 4 mmol) in dry ether at 0 °C was added lithium aluminum deuteride (340 mg, 8 mmol). After stirring at 25 °C for 8 hours, the reaction mixture was poured into cold 3 M HCl. The aqueous solution was then extracted with ethyl ether twice. The organic layers were combined, washed with 10 brine and dried over  $\text{MgSO}_4$ . Removal of the solvent gave 4- $\text{d}_5$  ethoxy  $\text{d}_2$ -phenethyl alcohol (680 mg, 98%) as a white solid.

15  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 2.79 (2H, s), 6.85 (2H, d,  $J = 8.5$  Hz), 7.13 (2H, d,  $J = 8.5$  Hz).

Scheme 12 displays a table of 7 different tags by combination of the compound in the horizontal column with the compound in the corresponding vertical column.

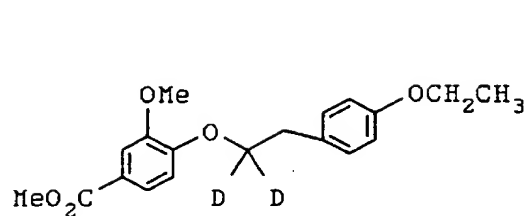
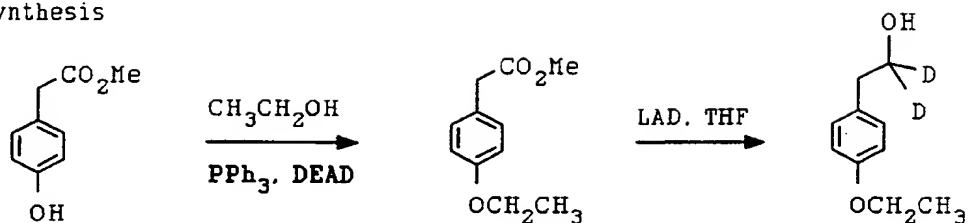
-137-

## Scheme 12

Mass Tag

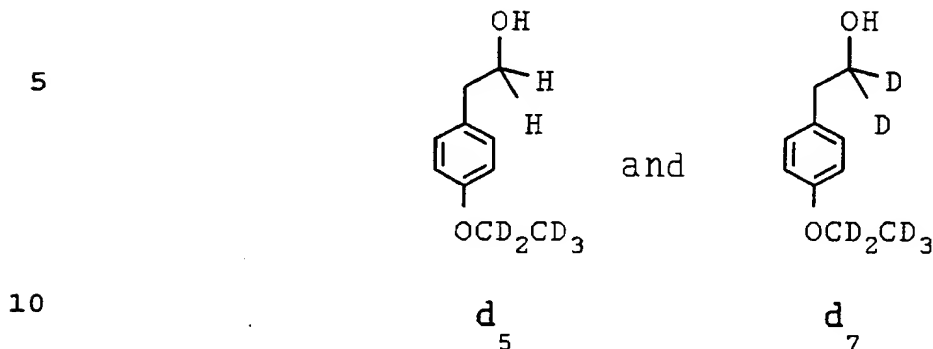
	$\text{CH}_3\text{CH}_2\text{OH}$	$\text{CD}_3\text{CH}_2\text{OH}$	$\text{CD}_3\text{CD}_2\text{OH}$
	0	3	5
	1	4	6
	2	5	7

Synthesis



Mass=M+2

-138-

Assessment of d-7 or d-5 deuterium samples

Sensitivity requires that the samples are derivatized to form the trimethyl silyl esters using derivatizing agent bis(trimethylsilyl)trifluoroacetamide (BSTFA) quantitatively for 30 minutes at room temperature. The reagent and solvent are removed at room temperature under a stream of  $\text{N}_2(\text{g})$ . The samples are resolvated with ethyl acetate and injected on column for analysis using positive chemical ionization mass spectrometry. The d-7 and d-5 components cannot be separated on the GC but mass spectrometry allows the ratio of the two components present to be determined when approximately 20 ng is injected on column using the Hewlett-Packard GC/MS. The  $(\text{M}+\text{NH}_4)^+$  ion observed in Figure 6 for the mass spectrum of the derivatized d-5 and d-7 sample show a  $m/z = 263$  for d-7 and  $m/z = 261$  for d-5 which agree with the calculated ratio. Fragments for the para-ethoxy-benzyl cation are also observed in Figure 6 at  $m/z = 156$  for d-7 and  $m/z = 154$  for d-5. Sensitivity may be increased to 1 ng or less injected on column if the more sensitive JEOL SX-102 high resolution mass spectrometer were used. The JEOL SX-102 is about 2 orders of magnitude more sensitive than the Hewlett-Packard system. Figure 6 shows spectra and chromatograms showing the improvement in chromatography due to derivatization. Figure 5 shows mass spectrum and chromatogram for the underivatized d-7 sample only.



-139-

It is evident from the above description that the subject invention provides a versatile, simple method for identifying compounds, where the amount of compound present precludes any assurance of the ability to obtain an accurate determination of its reaction history. The method allows for the production of extraordinarily large numbers of different products, which can be used in various screening techniques to determine biological or other activity of interest. The use of tags which are chemically inert under the process conditions allows for great versatility in a variety of environments produced by the various synthetic techniques employed for producing the products of interest. The tags can be readily synthesized and permit accurate analysis, so as to accurately define the nature of the composition.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

-140-

WHAT IS CLAIMED IS:

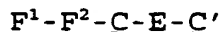
1. A method for recording the reaction history of a reaction series on each of a plurality of unique solid supports, wherein said reaction series involves at least two stages requiring differing agents or reaction conditions resulting in a different modification as to a plurality of said unique solid supports, resulting in a plurality of different final products on different unique solid supports, employing a combination of identifiers for recording said reaction history, said identifiers characterized by defining the choice of agent or reaction condition and the stage in said reaction series and being capable of being analyzed as to the choice and stage, said method comprising:
- reacting, at a first or intermediate stage of said series, a different agent or employing a different reaction condition with each of a group of said unique solid supports, said group comprising at least one of said unique solid supports, and a combination of identifiers wherein said combination of identifiers defines the choice of agent and the stage in said series as to each group of said unique solid supports, each of said identifiers being individually bound to said unique solid support directly or through other than a prior identifier;
- mixing said groups together and then dividing said plurality of unique solid supports into a plurality of groups for a second intermediate or final stage; and
- repeating said reacting at least once to provide a plurality of final products, having different products on the different individual unique solid supports.

-141-

2. A method according to claim 1, wherein at least 100 unique solid supports and at least 2 groups are employed in each said reacting.
- 5 3. A method according to claim 1, including the additional stages of screening said final products on said unique solid supports for a characteristic of interest; and identifying the reaction history of at least one final product having said characteristic of  
10 interest.
4. A method of claim 1, further comprising cleaving the final product from the solid support and screening said final product.  
15
5. A method of claim 1, further comprising treating the identifiers so as to detach tag components from the solid supports and reacting said tag components with a moiety capable of detection by fluorescence or  
20 electron capture.
6. A method of claim 5, wherein the detaching is done photochemically or oxidatively and the detectable moiety is derived from dansyl chloride or a  
25 polyhalobenzoylhalide.
7. A method of claim 1, wherein the identifier comprises tag components which have two characteristics, a characteristic capable of separation and a  
30 characteristic capable of detection.
8. A method according to claim 7, wherein said characteristic capable of detection is the ability to be detected by electron capture.  
35

-142-

9. A method according to claim 7, wherein said characteristic capable of detection is the ability to be detected by mass spectroscopy.
- 5 10. A method of claim 9, wherein the mass spectroscopy detects the presence of deuterium atoms.
11. A method according to claim 7, wherein said characteristic capable of detection is radioactivity.
- 10 12. A method according to claim 7, wherein said characteristic capable of detection is fluorescence.
13. A method according to claim 7, wherein said tags may be separated by means of chromatography.
- 15 14. A kit comprising a plurality of different separated organic compounds, each of the compounds characterized by having a distinguishable composition, encoding at least one bit of different information which can be determined by a physical measurement and sharing at least one common functionality.
- 20 15. A kit of claim 14, comprising at least 4 different functional organic compounds.
- 25 16. A kit according to claim 14, wherein said functional organic compounds are of the formula:
- 30



where  $F^1-F^2$  is a linker which allows for attachment to and detachment from a solid particle; and

35  $\underline{C}-E-\underline{C}'$  is a tag which can be determined by a physical measurement.

-143-

17. A kit according to claim 16, wherein said functional organic compounds differ by the number of methylene groups and/or halogens, nitrogens or sulfurs present.
- 5 18. A kit according to claim 16, wherein the C-E-C' portion can be removed photochemically.
- 10 19. A kit according to claim 16, wherein the C-E-C' portion can be removed oxidatively, hydrolytically, thermolytically, or reductively.
- 15 20. A solid support characterized by having a ligand bound thereto and having a combination of identifiers bound to said solid support.
- 20 21. A solid support according to claim 20, wherein said ligand is an oligomer which is an oligopeptide, oligonucleotide, oligosaccharide, polylipid, polyester, polyamide, polyurethane, polyurea, polyether, poly (phosphorus derivative) which is a phosphate, phosphonate, phosphoramidate, phosphonamides, phosphite, or phosphinamide, poly (sulfur derivative) which is a sulfone, sulfonate, sulfite, sulfonamide, or sulfenamide, where for the phosphorous and sulfur derivatives the indicated heteroatom for the most part will be bonded to C, H, N, O or S, and combinations thereof.
- 25 22. A solid support according to claim 20, wherein said ligand is a non-oligomer which is heterocyclic, aromatic, alicyclic, or aliphatic, and combinations thereof.
- 30 23. A solid support of claim 22, wherein the non-oligomer is a diazabicyclic, an azatricyclic, or a branched amide compound.
- 35

-144-

24. A solid support of claim 20, wherein the ligand is linked to the support through a non-labile linkage.
- 5 25. A solid support of claim 20, wherein the ligand is linked to the support through a cleavable linkage.
- 10 26. A solid support according to claim 20, wherein the identifier comprises tags, the tags being radioisotopes, or haloalkyl or haloarylallyl containing compounds.
- 15 27. A solid support of claim 20, which is a bead of about 10-2000  $\mu\text{m}$  in diameter, and wherein the identifiers comprise tag components which after cleavage from the bead can be separated by gas chromatography and or liquid chromatography detected by electron capture, mass spectroscopy, fluorescence, or atomic emission techniques.
- 20 28. A library comprising a plurality of solid supports according to claim 23.
- 25 29. A library of claim 28, wherein the final products have been cleaved from the solid support.
- 30 30. A library of claim 29, wherein the final products are a diazabicyclic, azatricyclic, or branched amide compounds.
- 35 31. A process for identifying compounds having a characteristic of interest which comprises screening a library of claim 28.
32. A process of claim 31, wherein the compounds have been cleaved from the solid surface.

-145-

33. A process of claim 32, wherein the compound is a diazabicyclic, azatricyclic, or branched amide compound.

5 34. A method for producing a ligand involving a reaction series employing a method for recording the reaction history of a reaction series on each of a plurality of unique solid supports, wherein said reaction series involves at least two stages requiring  
10 differing agents and/or reaction conditions resulting in a different modification as to a plurality of said unique solid supports, resulting in a plurality of different final products on different unique solid supports, employing a combination of identifiers for  
15 recording said reaction history, said identifiers characterized by defining the choice of agent or reaction condition and the stage in said series and being capable of being analyzed as to the choice and stage, said method comprising:  
20 reacting, at a first or intermediate stage of said series, a different agent or employing a different reaction condition with each of a group of said unique solid supports, said group comprising at least one of said unique solid supports, and a combination  
25 of identifiers wherein said combination of identifiers defines the choice of agent and the stage in said series as to each group of said unique solid supports, each of said identifiers being individually bound to said unique solid support directly or  
30 through other than a prior identifier;  
mixing said groups together and then dividing said plurality of unique solid supports into a plurality of groups for a second intermediate or final stage;  
repeating said reacting at least once to provide a  
35 plurality of ligands, having different products on the different individual unique solid surfaces; and

-146-

identifying said reaction history of at least one selected unique solid surface by means of said combination of identifiers.

- 5      35. A ligand according to claim 34, wherein said  
identifying includes the stage of screening said  
ligands for a characteristic of interest.
- 10      36. A method for producing a ligand involving a reaction  
series employing a method for recording the reaction  
history of a reaction series on each of a plurality  
of unique solid surfaces, wherein said reaction  
series involves at least two stages requiring  
15      differing agents and/or reaction conditions resulting  
in a different modification as to each of a plurality  
of said unique solid surfaces, resulting in a  
plurality of different ligands on different unique  
solid surfaces, employing combinations of identifiers  
for recording said reaction history, said combination  
20      of identifiers characterized by defining the choice  
of agent and/or reaction condition and the stage in  
said series and being capable of being analyzed as to  
the choice and stage, said method comprising:  
reacting, at a first or intermediate stage of said  
25      series, a different agent and/or employing a  
different reaction condition with each of a group of  
said unique solid surfaces, said group comprising at  
least one of said unique solid surfaces, and a  
combination of identifiers wherein said combination  
30      of identifiers defines the choice of agent and the  
stage in said series as to each group of said unique  
solid surfaces, each of said identifiers being  
individually bound to said unique solid surface  
through other than a prior identifier by a cleavable  
35      link;

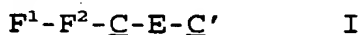


-147-

5 mixing said groups together and then dividing said plurality of unique solid surfaces into a plurality of groups for a second intermediate or final stage; repeating said reacting to provide a plurality of ligands having different ligands on the different individual unique solid surfaces; screening the ligands from a plurality of each of said unique solid surfaces for a characteristic of interest; and  
10 identifying said reaction history of at least one selected unique solid surface having ligand having said characteristic of interest by detaching the tag members from said unique solid surface and identifying said tag members by means of a differing  
15 characteristic.

37. A method according to claim 36, wherein said tags differ in an homologous series and are detected by electron capture gas chromatography or mass  
20 spectroscopy.

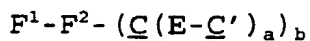
38. A compound of the Formula I:



25 where  $F^1-F^2$  is a linker which allows for attachment to and detachment from a support; and  $\underline{C}-E-\underline{C}'$  is the tag which is capable of analysis; E is a tag component which allows for detection, or allows for detection and provides for separation as a result of variable substitution;  
30  $\underline{C}$  and  $\underline{C}'$  are tag components which allow for individual detection;  $F^2$  is a linking component capable of being selectively cleaved to release the tag components; and  $F^1$  is a functional group which allows ready attachment  
35 of the compound to a synthesis support.

-148-

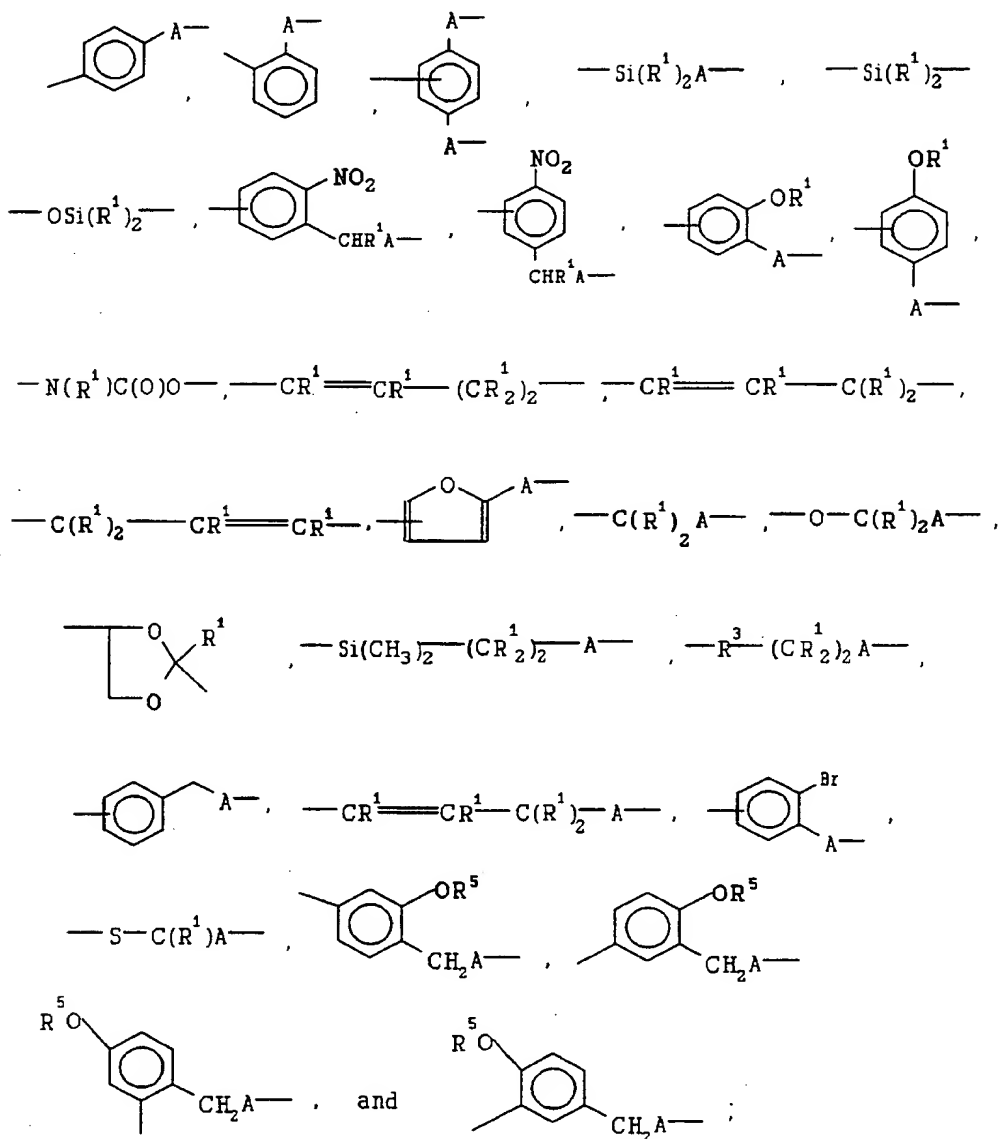
39. A compound of claim 38, having the formula:

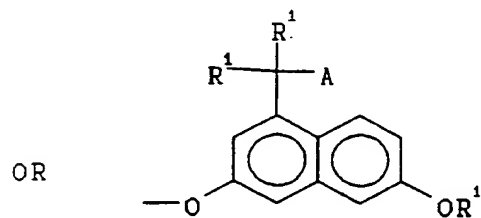
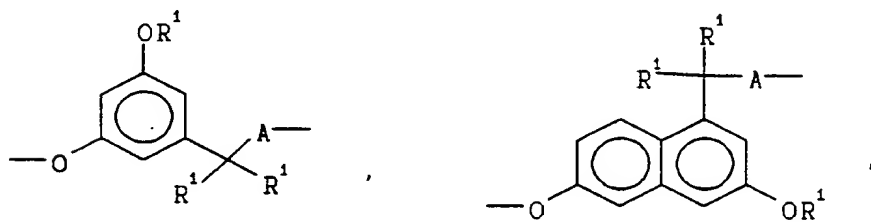
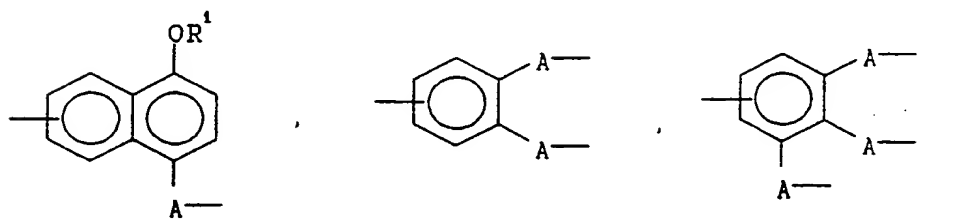
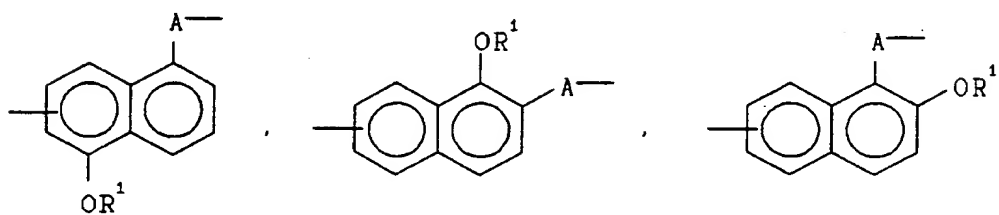
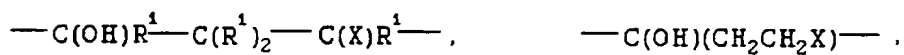
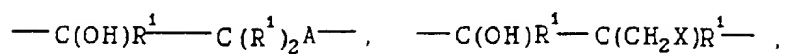


wherein:

5  $F^1$  is  $\text{CO}_2\text{H}$ ,  $\text{CH}_2\text{X}$ ,  $\text{NR}^1\text{R}^1$ ,  $\text{C}(\text{O})\text{R}^1$ ,  $\text{OH}$ ,  $\text{CHN}_2$ ,  $\text{SH}$ ,  $\text{C}(\text{O})\text{CHN}_2$ ,  $\text{S}(\text{O}_2)\text{Cl}$ ,  $\text{S}(\text{O}_2)\text{CHN}_2$ ,  $\text{N}_3$ ,  $\text{NO}_2$ ,  $\text{NO}$ ,  $\text{S}(\text{O}_2)\text{N}_3$ ,  $\text{OC}(\text{O})\text{X}$ ,  $\text{C}(\text{O})\text{X}$ ,  $\text{NCO}$ , or  $\text{NCS}$ ;

$F^2$  is





-150-

with the proviso that when  $F^2$  is a bond,  $F^1$  is OH or COOH;

A is -O, -OC(O)O-, -OC(O)-, or -NHC(O)-;

5 C is a bond,  $C_1$ - $C_{20}$  alkylene optionally substituted by 1-40

F, Cl, Br,  $C_1$ - $C_6$  alkoxy,  $NR^4R^4$ ,  $OR^4$ , or  $NR^4$ , or  
-[( $C(R^4)_2$ )<sub>m</sub>-Y-Z-Y-( $C(R^4)_2$ )<sub>n</sub>Y-Z-Y]<sub>p</sub>-; with the proviso  
that the maximum number of carbon atoms in C+C' is  
20;

10 C' is H; F; Cl;  $C_1$ - $C_{20}$  alkylene optionally substituted  
by

1-40 F, Cl, Br,  $C_1$ - $C_6$  alkoxy,  $NR^4R^4$ ,  $OR^4$ , or  $NR^4$ , or  
-[( $C(R^4)_2$ )<sub>m</sub>-Y-Z-Y-( $C(R^4)_2$ )<sub>n</sub>Y-Z-Y]<sub>p</sub>-; with the proviso  
that the maximum number of carbon atoms in C+C' is  
15 20;

E is  $C_1$ - $C_{10}$  alkyl substituted by 1-20 F, Cl or Br; or  
Q-aryl

wherein the aryl is substituted by 1-7 F, Cl,  $NO_2$ ,  
20  $SO_2R^5$ , or substituted phenyl wherein the substituent  
is 1-5 F, Cl,  $NO_2$ , or  $SO_2R^5$ ;

E-C' may be -H, -OH, or amino;

$R^1$  is H or  $C_1$ - $C_6$  alkyl;

$R^3$  is C=O, C(O)O, C(O)NR<sup>1</sup>, S, SO, or  $SO_2$ ;

$R^4$  is H or  $C_1$ - $C_6$  alkyl;

25  $R^5$  is  $C_1$ - $C_6$  alkyl;

a is 1-5;

b is 1-3;

m and n is each 0-20;

p is 1-7;

30 Q is a bond, O, S,  $NR^4$ , C=O, -C(O)NR<sup>5</sup>, -NR<sup>5</sup>C(O)-, -  
C(O)O-,

or -OC(O)-;

X is a leaving group such as Br, Cl, triflate,  
mesylate,

35 tosylate, or OC(O)OR<sup>5</sup>;

Y is a bond, O, S, or  $NR^4$ ;

-151-

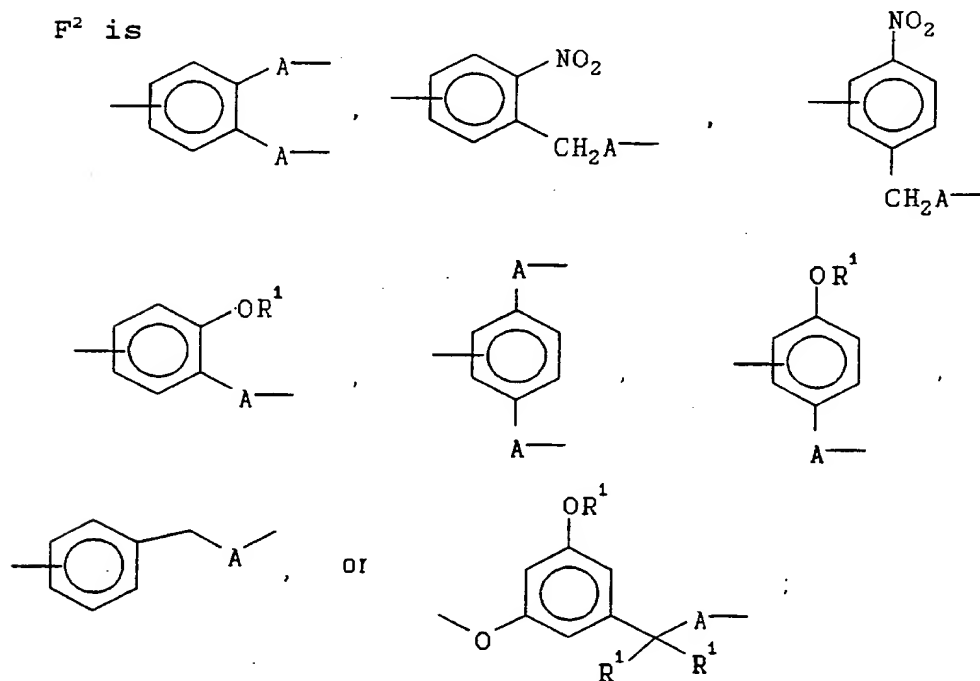
Z is a bond; phenyl optionally substituted by 1-4 F, Cl, Br, C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> alkoxy, C<sub>1</sub>-C<sub>6</sub> alkyl substituted by 1-13 F, Cl, or C<sub>1</sub>-C<sub>6</sub> alkyloxy substituted by 1-13 F, Cl, or Br; (C(R<sup>4</sup>)<sub>2</sub>)<sub>1-20</sub>; or (CF<sub>2</sub>)<sub>1-20</sub>; with the proviso that when Z is a bond one of its adjacent Y's is also a bond and aryl is a mono- or bi-cyclic aromatic ring containing up to 10 carbon atoms and up to 2 heteroatoms selected from O, S, and N.

40. A compound of claim 39, wherein:

F<sup>1</sup> is

CO<sub>2</sub>H, OH, CHN<sub>2</sub>, C(O)CHN<sub>2</sub>, C(O)X, NCS, or CH<sub>2</sub>X;

F<sup>2</sup> is



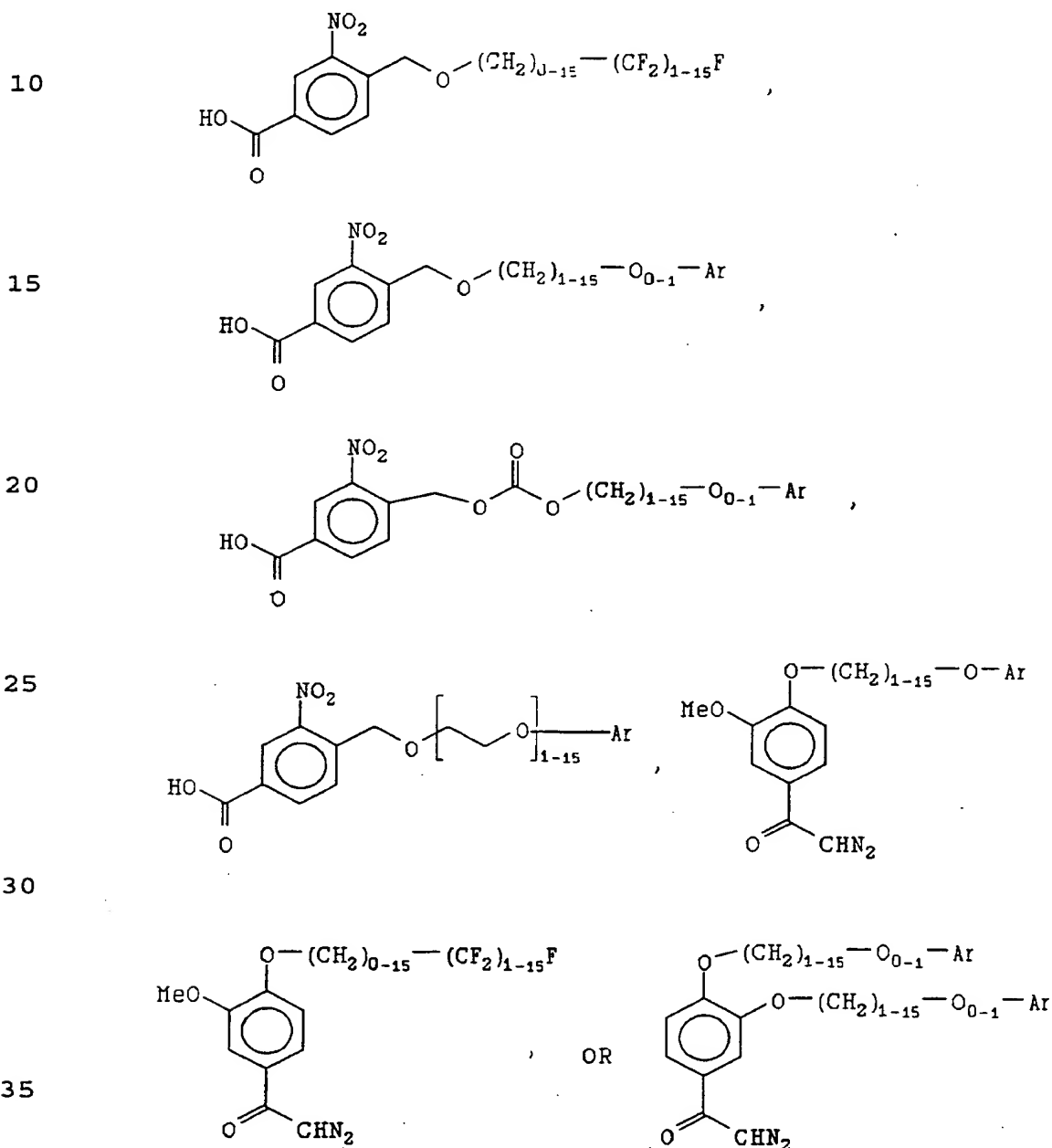
C and C' is each independently C<sub>1</sub>-C<sub>20</sub> alkylene unsubstituted or substituted by 1-40 F or Cl, or [O-(CH<sub>2</sub>)<sub>2-3</sub>]<sub>p</sub>;

-152-

E is C<sub>1</sub>-C<sub>10</sub> alkyl substituted by 1-20 F or Cl; Q-aryl where aryl is a bi-cyclic aromatic ring substituted by 1-7 F or Cl; or Q-phenyl substituted by 1-5 F, Cl, NO<sub>2</sub>, or SO<sub>2</sub>R<sup>5</sup>; and

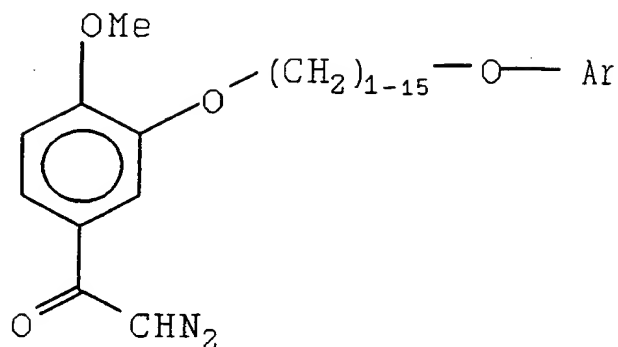
5 Q is a bond, O, -NR<sup>5</sup>C(O)-, or -OC(O)-.

41. A compound of claim 39, having the formula:



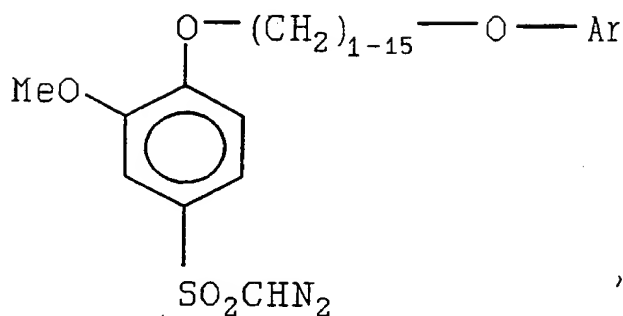
-153-

5



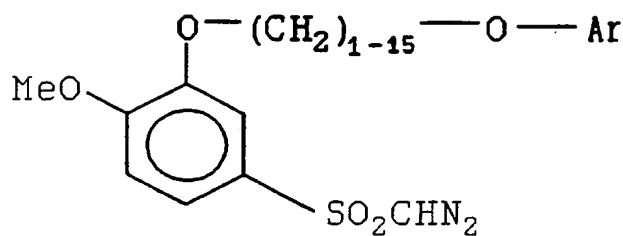
10

15



20

25



30

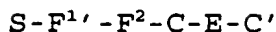
wherein Ar is pentafluoro-, pentachloro-, or pentabromophenyl, 2,3,5,6-tetrafluoro-4(2,3,4,5,6-pentafluorophenyl)phenyl, 2,4,6-trichlorophenyl, 2,4,5-trichlorophenyl, 2,6-dichloro-4-fluorophenyl, or 2,3,5,6-tetrafluorophenyl.

35

42. A compound of claim 39, wherein:  
E-C' is H, OH, or NH<sub>2</sub>.

-154-

43. A composition of the formula



wherein:

S is a soluble or solid support;

$\underline{C}-E-\underline{C}'$  is the tag which is capable of analysis where E is a tag component which (a) allows for detection, such as an electrophoric group which can be analyzed by gas chromatography or mass spectroscopy or (b) allows for detection and for separation as a result of variable substitution;

$\underline{C}$  and  $\underline{C}'$  are tag components which allow for distinguishing one tag from all other tags, usually allowing for separation as a result of variable length or substitution, for example, varying the chromatographic retention time or the mass spectroscopy ratio Z/e;

$F^2$  is a linking component capable of being selectively cleaved to release the tag; and

$F^{1'}$  is a functional group which provides for attachment to the support.

44. A composition of claim 43, wherein:

S is a capillary, hollow fiber, needle, solid fiber, cellulose bead, pore-glass bead, silica gel, polystyrene bead optionally cross-linked with divinylbenzene, grafted co-poly bead, poly-acrylamide bead, latex bead, dimethylacrylamide bead optionally cross-linked with N,N'-bis-acryloyl ethylene diamine, glass particles coated with a hydrophobic polymer, or low molecular weight non-cross-linked polystyrene; and

$F^{1'}-F^2-\underline{C}-E-\underline{C}'$  is the residue of Formula I attached to S.

45. The method of claim 1, wherein the combination of identifiers defines a binary coding scheme.

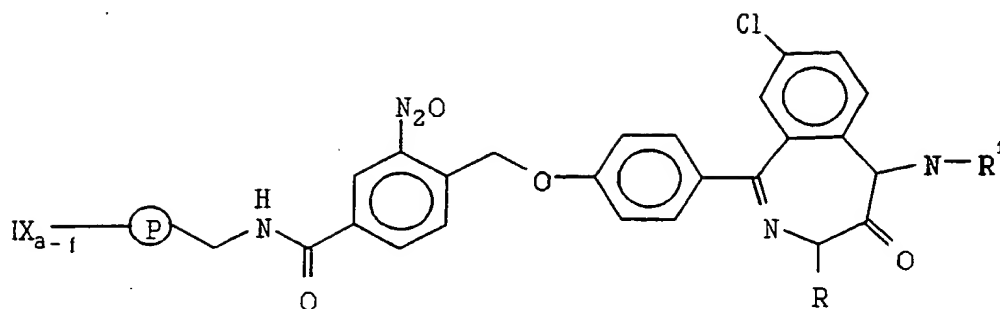


-155-

46. The method of claim 1, wherein the identifiers are of claim 38.
47. The method of claim 1, wherein the identifiers are of claim 39.
48. The method of claim 1, wherein the identifiers are of claim 40.
49. The method of claim 1, wherein the identifiers are of claim 42.
50. The method of claim 1, further comprising detaching the tag members from said unique solid surfaces.
51. The method of claim 50, wherein the tag members are detached photochemically, oxidatively, hydrolytically, thermolytically, or reductively.
52. The method of claim 1, further comprising detaching non-oligomer ligands from said unique solid surfaces photochemically.

-156-

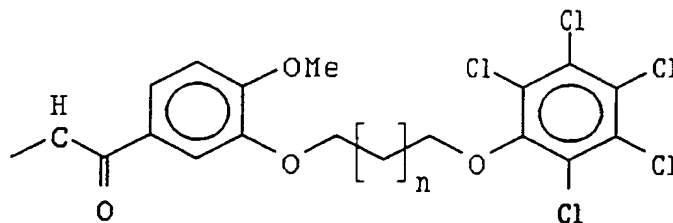
53. A compound of the formula



wherein:

P is a polystyrene resin;

IX<sub>a-f</sub> is a plurality of residues of the formula



wherein:

n is 1 - 6;

R is CH<sub>3</sub>, CH(CH<sub>3</sub>)<sub>2</sub>, CH<sub>2</sub>CO<sub>2</sub>H, (CH<sub>2</sub>)<sub>4</sub>NH<sub>2</sub>, CH<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>-OH, or CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>; and

R<sup>1</sup> is H, CH<sub>3</sub>, C<sub>2</sub>H<sub>5</sub>, CH<sub>2</sub>CH=CH<sub>2</sub>, or CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>.

54. A method of synthesizing a chemical compound so that the structure of the compound is readily determinable, which comprises synthesizing the compound on the surface of a solid support under conditions such that the solid support at the completion of the synthesis of the compound has bound to it a plurality of identifiers which encode the reaction stages associated with the synthesis of the compound.

-157-

55. A method of synthesizing a library of chemical compounds so that the structure of each compound in the library is readily determinable which comprises synthesizing each compound on the surface of a unique solid support under conditions such that each such unique support at the completion of the synthesis of the library of compounds has bound to it a plurality of identifiers which encode the reaction stages associated with the synthesis of the compound synthesized on such solid support.
56. A method of determining the structure of a chemical compound which comprises synthesizing the compound by the method of claim 54 or 55, isolating the solid support upon which the compound was synthesized, treating the solid support so isolated so as to cause the tag components of each of the identifiers bound to the solid support to be released, determining the identity or quantity or both of each tag component so released, and deriving the structure of the compound from the identities or quantities or both of all such tag components.
57. A method of identifying a compound having a desired characteristic which comprises synthesizing a library of chemical compounds by the method of claim 55, separately testing each of the compounds in the resulting library in an assay which identifies compounds having the desired characteristic so as to identify any compounds present in the library which has the desired characteristic.
58. A method of claim 57, further comprising determining the structure of the compound so identified.
59. A library of chemical compounds, each compound in the library being bound to a unique solid support and

-158-

each such solid support having bound to it a plurality of identifiers which encode the reaction stages associated with the synthesis of the compound bound to such solid support.

60. A library of claim 59, wherein compounds in the library are diazabicyclic compounds.
61. A library of claim 59, wherein compounds in the library are azatricyclic compounds.
62. A library of claim 59, wherein compounds in the library are branched amide compounds.
63. A library of claim 59, wherein compound in the library are peptides.
64. A method of identifying a compound having a desired characteristic which comprises testing a library of chemical compounds according to claim 58 in an assay which identifies compounds having the desired characteristic so as to identify any compound present in the library which have the desired characteristic.
65. A method of claim 64, further comprising determining the structure of the compound so identified.
66. A compound identified by the method of claim 64.
67. A method of claim 64, wherein the desired characteristic is antagonism for the human neurokinin 1/bradykin receptor and the library of chemical compounds comprises azatricyclic compounds.
68. A method of claim 64, wherein the desired characteristic in usefulness as a muscle relaxant, a

-159-

tranquilizer or a sedative and the library of chemical compounds comprises bezodiazepines.

- 5 69. A method of claim 64, wherein the desired characteristic is useful in the treatment of hypertension or Raynaud's syndrome and the library of chemical compounds comprises branched amides.
- 10 70. A composition comprising at least 6 different components, said components being associated in a kit or in a common medium, each component having a distinguishable moiety, said components being characterized by:
- 15 each distinguishable moiety being substantially chemically inert, differing in molecular weight, and having an identifiable characteristic different from each of the other distinguishable moieties other than light emission;
- 20 each moiety joined to a linking group having an active functionality capable of forming a covalent bond, through a linking unit to individually separable solid surfaces, or joined to a group which
- 25 is detectable at less than 1 nanomole,
- with the proviso that when said moieties are joined to said linking group, said components are physically segregated.
- 30 71. A composition according to claim 70, wherein said solid supports are beads.
- 35 72. A composition according to claim 70, wherein each component comprises molecules of different compounds bound to individual separable solid surfaces, wherein said distinguishable moieties encode the synthetic

-160-

protocol used to synthesize said molecules on said solid surfaces.

73. A composition according to claim 70, wherein said distinguishable moieties define an homologous series and/or a series of substituents on a core molecule.

74. A composition comprising at least 6 different markers said markers being associated in a kit or in a common medium, each marker having a distinguishable moiety which is substantially chemically inert, differing from each other in molecular weight, said markers of the formula:

(1)  $\Delta - \{ \Delta - (T)_\alpha \text{ or } (T)_\alpha - \Delta \text{ or } \Delta^1 - (T)_\alpha - \Delta^2 \}$

wherein

$\Delta$  is a linking group comprising a functionality for bonding to a solid support and a functionality for detachment from the solid support, which may be included in the functionality for bonding to the solid support;

$\Delta$  is a distinguishing group, which allows for the distinguishing by a physical characteristic of each of the markers from each of the other markers by other than fluorescence, so as to provide a set of markers which allows for coding a multistep synthetic procedure, and includes any remaining functional group after detachment, which was previously associated with the linked group;

$\Delta^1$  and  $\Delta^2$  are portions of the distinguishing group, which together define a distinguishing group and when joined together come within the definition of  $\Delta$ ;

-161-

T is a detectable group, which when attached to the distinguishing group allows for the low level detection of the marker, where the detectable group may be present on the marker in the kit or may be added later to the distinguishing group and if attached to the linking group, includes any remaining functional group after detachment, which was previously associated with the linking group; and

5

10

$\alpha$  is 0 or 1, indicating that the detectable group may or may not be present;

$$(2) \quad ss-(\Delta'-\{\Delta-(T)_{\alpha} \text{ or } (T)_{\alpha}-\Delta \text{ or } \Delta^1-(T)_{\alpha}-\Delta^2\})_{\beta}$$

15

wherein:

all of the symbols have been defined previously, except as follows:

20

ss is a solid support;

$\Delta'$  is a linking group covalently bonded to ss; and

25

$\beta$  is an integer as to each solid support and is at least six and usually not more than about 30;

$$(3) \quad \Delta''-\{\Delta''-(T)_{\alpha} \text{ or } (T)_{\alpha}-\Delta \text{ or } \Delta'^1-(T)_{\alpha}-\Delta^2\}$$

wherein:

30

all of the symbols have been defined previously, except as follows:

35

$\Delta''$  is hydrogen or the residue of the linking group after photolytic cleavage, elimination or other chemical reaction which results in detachment from the solid support;

-162-

$\Delta''$  or  $\Delta'^1$  is  $\Delta$  or  $\Delta^1$  or a modified  $\Delta$  or  $\Delta^1$ , respectively, as a result of the detachment of the marker from the solid support;

5         $T''$  is  $T$  or a modified  $T$  as a result of the detachment of the marker from the solid support;

(4)  $T_\alpha - \Lambda'' - \{\Delta'' - (T)_\alpha$  or  $(T'')_\alpha - \Delta$  or  $\Delta'^1 - (T)_\alpha - \Delta^2\}$   
wherein:

10

all of the symbols have been defined previously and

$\Lambda''$  is a bond or the remaining portion of the linker group after attachment to  $T$ ;

15

with the additional limitation that only one  $\alpha$  is 1.

75. A composition according to claim 74, wherein the markers are of the formula (1).

20

76. A composition according to claim 74, wherein the markers are of the formula (2).

77. A composition according to claim 74, wherein the markers are of the formula (3).

25

78. A composition according to claim 74, wherein the markers are of the formula (4).

79. A composition according to claim 74, wherein  $\Delta$  is bound to  $\Lambda$ .

30

80. A compound library comprising at least one hundred unique solid supports characterized by:

35



-163-

each solid support having (1) an individual compound bound to said solid support as the major compound bound to said support; and

5 (2) a plurality of nonsequencable markers, where said markers are individual molecules which are physically distinguishable in being physically separable, differing in molecular weight and being distinguishable by other than fluorescence and are  
10 substituted so as to be detectable at less than about a nanomole or have a functional group for bonding to a substituent which is detectable at less than about a nanomole.

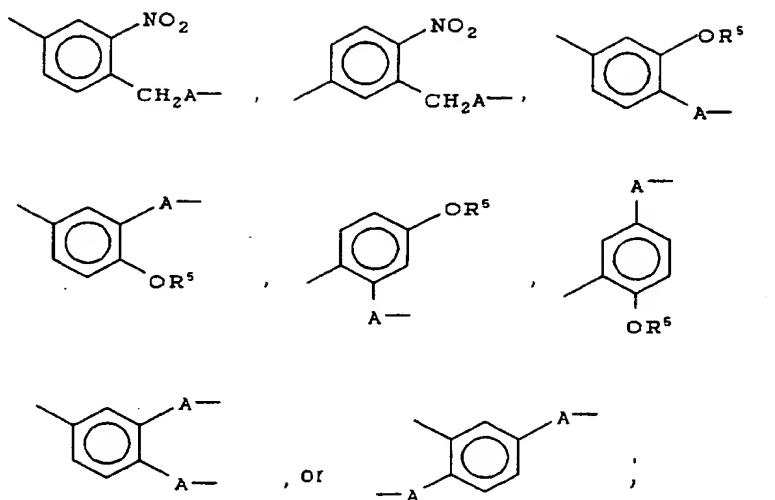
15 81. A compound library according to claim 80, wherein each solid support has at least about 6 markers.

82. A compound library according to claim 80, wherein said markers define a binary or higher code encoding  
20 the synthetic protocol used for synthesizing said compound on said solid support.

83. The compound of claim 38, wherein  $F^1$  is  $\text{CHN}_2$ ,  $\text{C(O)CHN}_2$ ,  $\text{S(O}_2\text{)CHN}_2$ ,  $\text{N}_3$ ,  $\text{NO}_2$ , or  $\text{SO}_2\text{N}_3$ .

25 84. The compound of claim 38, wherein  $F^1$  is  $\text{COOH}$ ,  $\text{CHN}_2$ ,  $\text{C(O)CHN}_2$ ,  $\text{S(O}_2\text{)CHN}_2$ ,  $\text{COCl}$ ,  $\text{OH}$ ,  $\text{SH}$ ,  $\text{CH}_2\text{X}$ , or  $\text{NHR}^1$ ;

-164-

wherein  $F^2$  is

wherein A is -O- or -OC(O)O-;

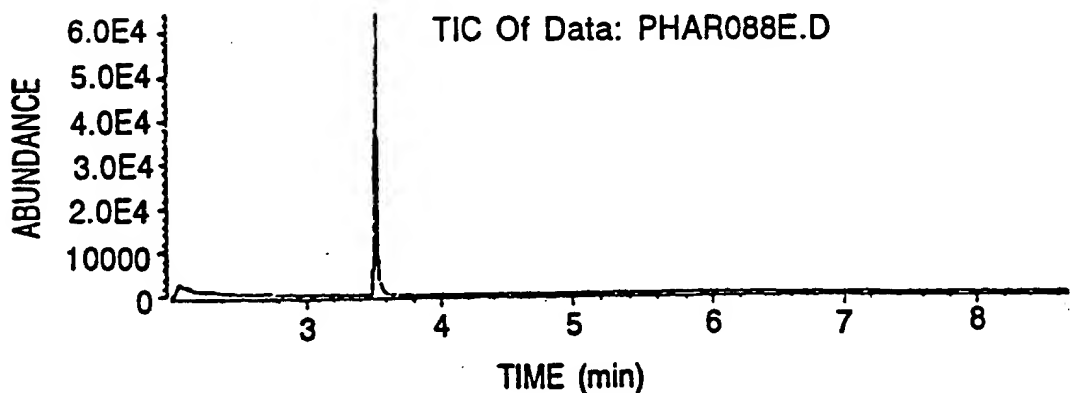
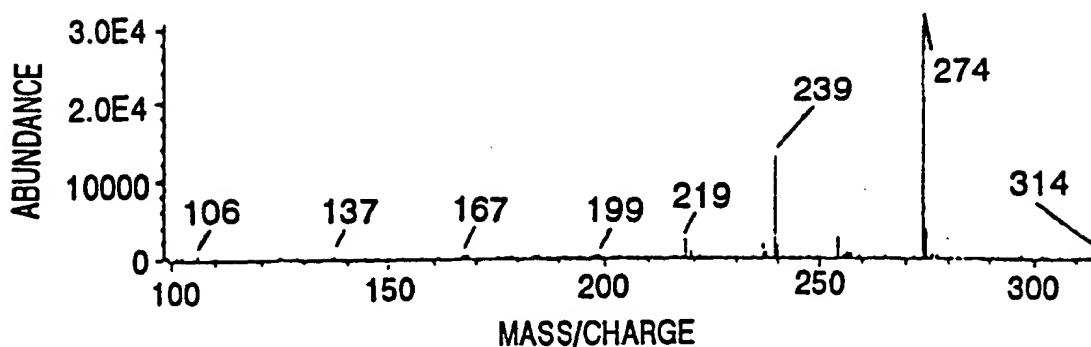
- 5  $\underline{C}(E-\underline{C}')_a$  is  $-(CR^4_2)_{1-15}-(O)_{0-1}-Ar$ ,  $-(CR^4_2)_{0-15}-(CF_2)_{1-15}-F$ ,  
 $-(CR^4_2)_{0-15}-(CF_2)_{1-15}-(CR^4_2)_{0-15}-H$ , or  
 $-(CH_2)_{1-20}-(O)_{0-1}-(CH_2)_{1-19}-(CH_2)_{0-24}-(O)_{0-1}-Ar$ ,

10 wherein Ar is pentafluoro-, pentachloro-, or pentabromophenyl, 2,3,5,6-tetrafluoro-4(2,3,4,5,6-pentafluorophenyl)phenyl, 2,4,6-trichlorophenyl, 2,4,5-trichlorophenyl, 2,6-dichloro-4-fluorophenyl, or 2,3,5,6-tetrafluorophenyl.

1/6

FIGURE 1

Scan 131(3.513min) Of Data: PHAR088E.D



Scan 131(3.513min) Of Data: PHAR088E.D  
 Pharmacopeia GC/CIMS CH<sub>4</sub>/NH<sub>3</sub>(g) 90/350 A/D=1

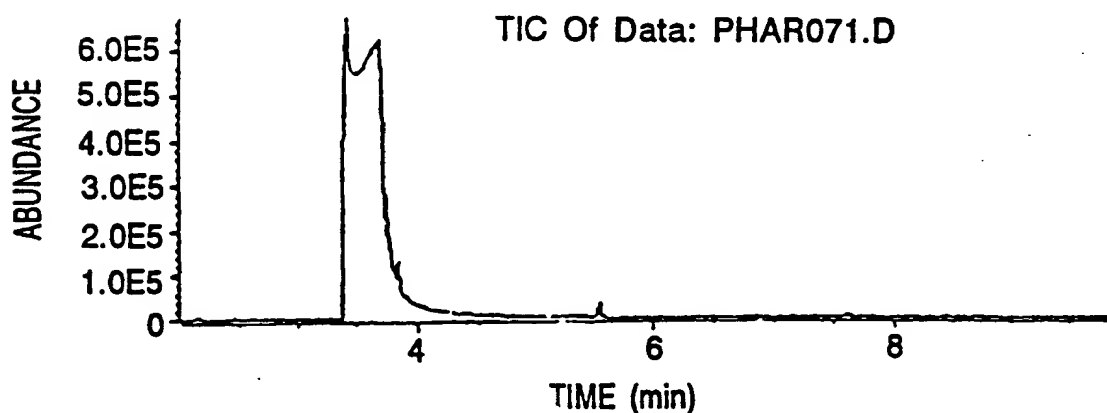
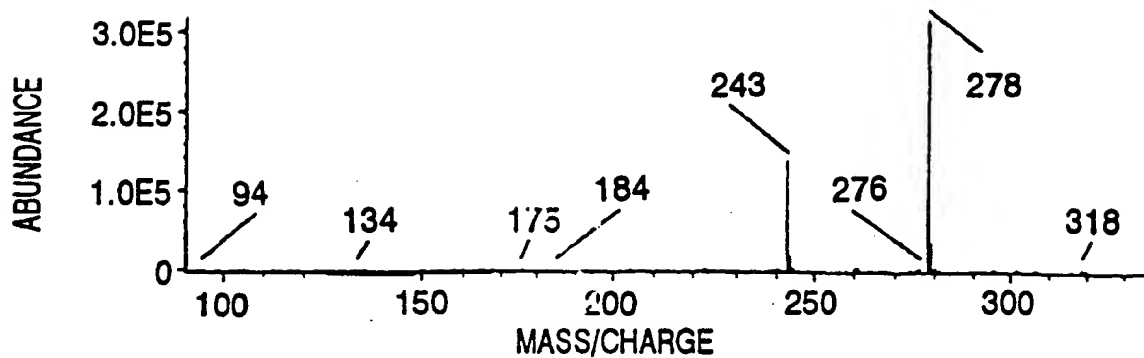
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
203.30	2	237.05	17	255.20	12	272.05	11
216.20	4	239.05	411	255.45	12	274.05	1000
218.05	70	240.05	48	256.20	23	275.05	111
219.20	25	241.20	4	257.05	15	276.20	11
221.20	5	247.30	5	259.05	6	277.20	3
233.95	2	254.20	84	265.05	8	297.15	4
236.20	52						

SUBSTITUTE SHEET (RULE 26)

2/6

FIGURE 2

Scan 124 (3.434 min) Of Data: PHAR071.D



Scan 124 (3.434 min) Of Data: PHAR071.D

Pharmacopeia GC/CIMS CH<sub>4</sub>/NH<sub>3</sub>(g) 90/350 A/D=1

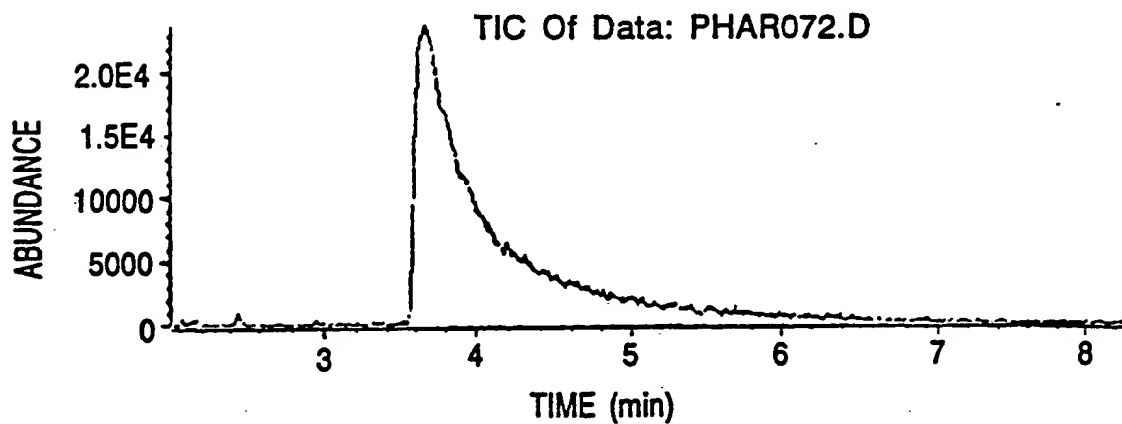
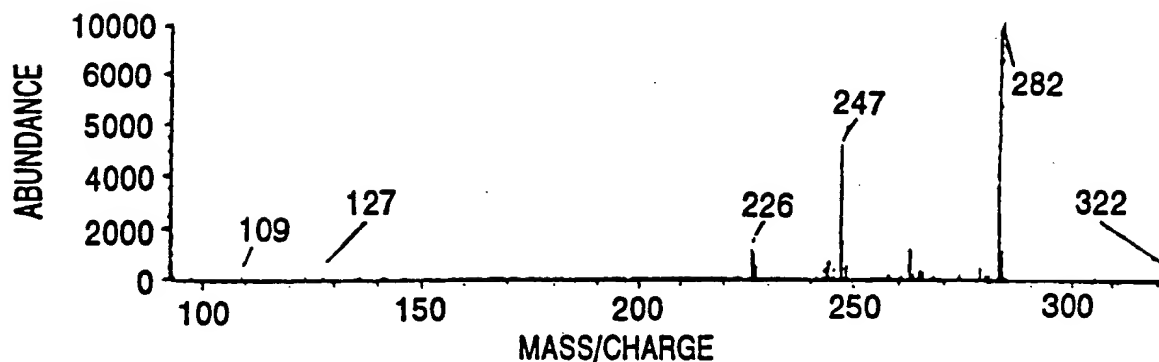
m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
203.05	1	238.05	1	260.20	11	279.05	113
210.20	1	239.20	2	261.05	13	280.05	10
213.20	1	240.20	8	262.20	2	281.05	1
220.20	1	241.20	7	263.20	1	289.20	1
222.05	8	243.05	429	269.05	1	290.05	2
223.05	3	244.05	47	274.05	2	291.05	1
224.20	1	245.05	3	275.30	4	292.05	1
224.55	1	251.20	1	276.20	13	295.15	3
225.30	1	258.20	9	278.05	1000		

SUBSTITUTE SHEET (RULE 26)

3/6

FIGURE 3

Scan 143 (3.648 min) Of Data: PHAR072.D



Scan 143 (3.648 min) Of Data: PHAR072.D

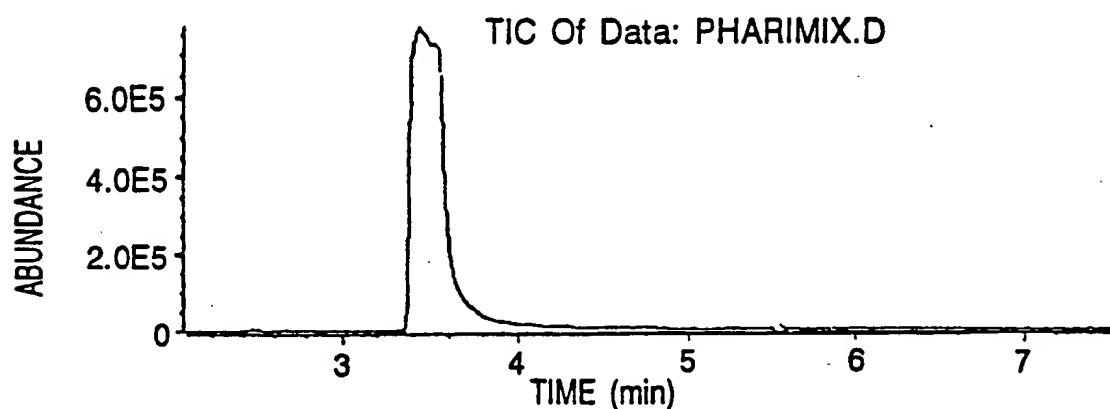
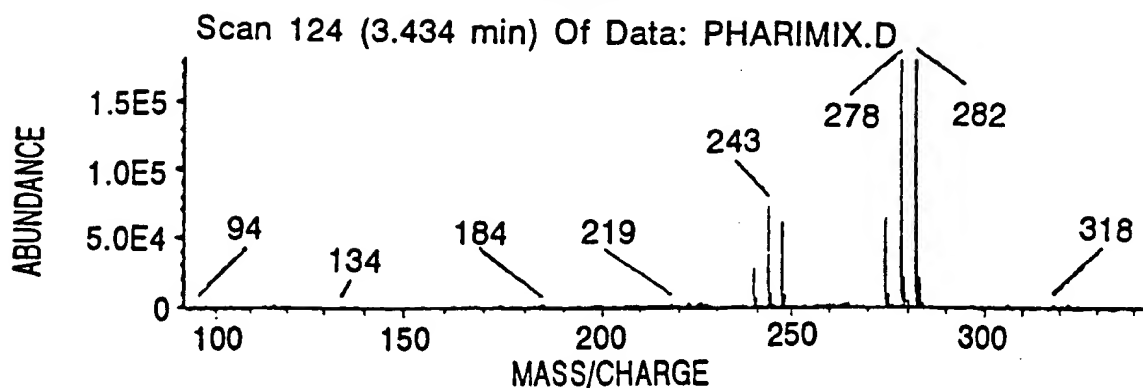
Pharmacopeia GC/CIMS CH<sub>4</sub>/NH<sub>3</sub>(g) 90/350 A/D=1

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
208.20	7	244.05	66	262.20	124	278.20	39
223.20	7	245.20	35	263.05	18	279.20	13
226.20	102	247.20	493	264.05	25	280.20	11
227.30	42	248.20	49	265.20	21	282.20	1000
241.20	9	257.95	12	267.20	7	283.20	115
242.95	34	260.55	13	273.20	12	284.20	9

SUBSTITUTE SHEET (RULE 26)

4/6

FIGURE 4



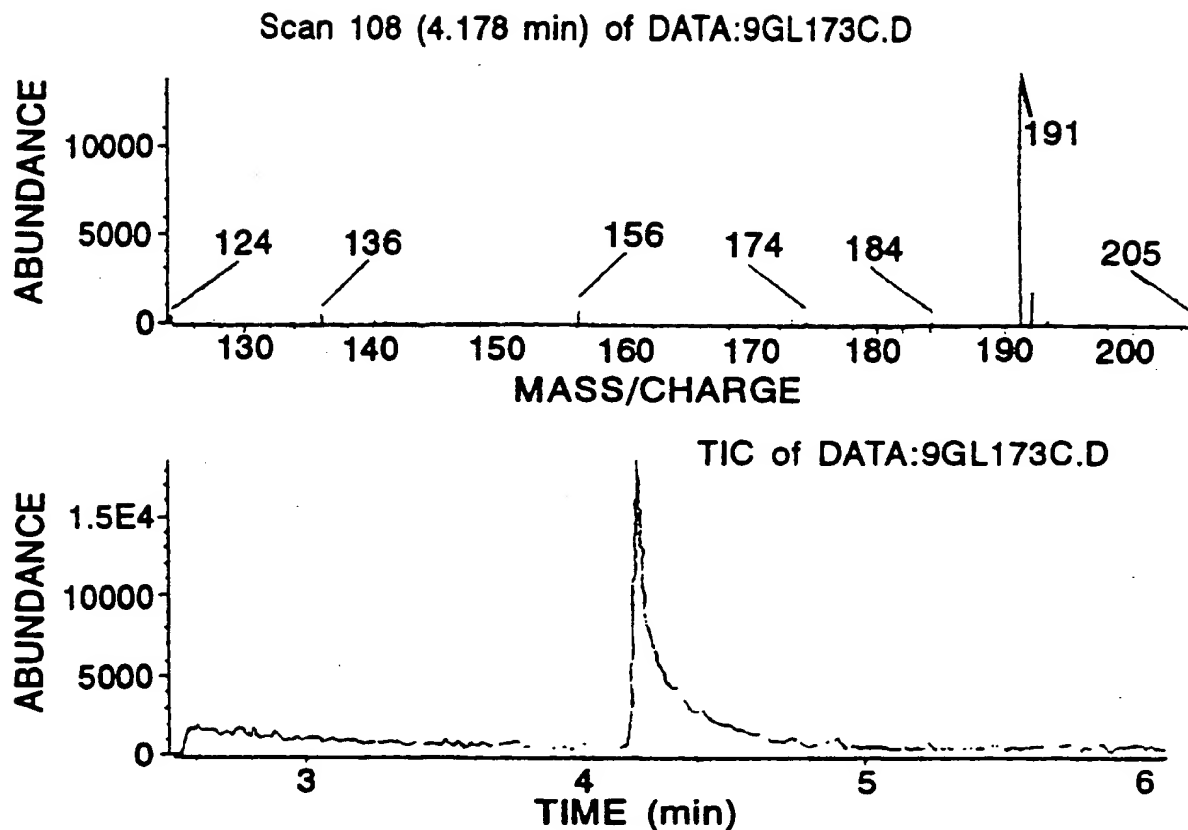
Scan 124 (3.434 min) Of Data: PHARIMIX.D  
Pharmacopeia GC/CIMS CH<sub>4</sub>/NH<sub>3</sub>(g) 90/350 A/D=1

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
203.05	1	237.20	2	261.05	13	283.20	112
206.95	1	239.05	178	262.05	9	284.05	11
210.30	1	240.05	27	263.20	3	285.05	1
213.05	1	241.05	8	264.20	12	285.95	1
213.95	1	243.05	409	265.20	13	288.30	1
218.05	4	244.05	49	266.05	2	290.05	1
219.20	2	245.05	7	267.20	1	291.20	2
220.05	1	247.05	402	268.30	1	292.05	2
222.05	9	248.05	44	269.05	1	293.40	1
223.05	3	249.20	4	274.05	432	293.75	1
224.20	2	251.20	1	275.05	51	294.40	2
225.20	2	254.05	5	276.05	18	295.25	3
226.20	9	256.20	6	278.05	996	296.25	2
227.05	3	257.05	7	279.05	112	297.25	2
228.20	1	258.20	12	280.20	18	298.15	1
229.20	1	259.20	3	282.20	1000	299.25	4
236.05	2	260.20	10				

SUBSTITUTE SHEET (RULE 26)

5/6

FIGURE 5



Scan 108 (4.178 min) of DATA:9GL173C.D

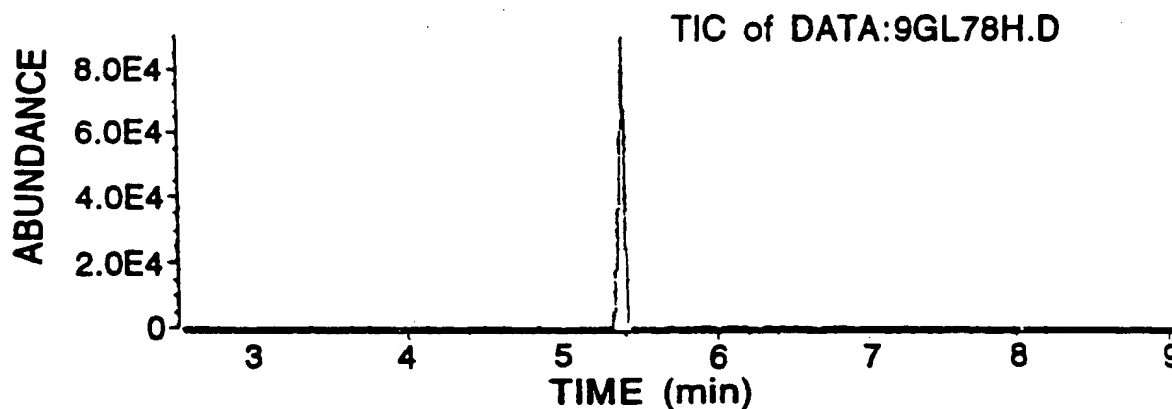
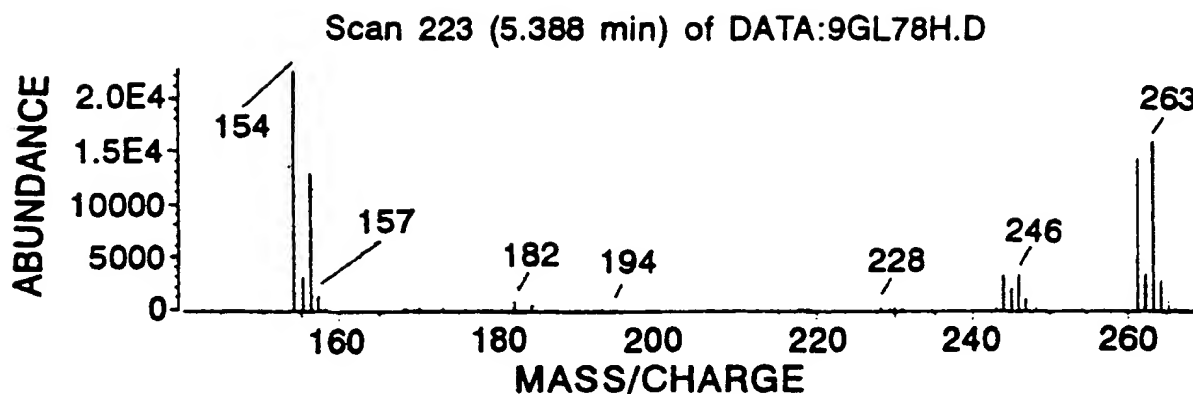
GE LI #1 DIL GC/CIMS CH4/NH3(g) 90/350 A/D=3

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
124.15	20	156.15	64	174.25	22	192.20	125
136.15	32	157.25	12	184.20	9	183.30	14
140.15	12	173.15	17	191.20	1000	205.20	4
141.00	6						

SUBSTITUTE SHEET (RULE 26)

6/6

FIGURE 6



Scan 223 (5.388 min) of DATA:9GL78H.D  
 GE LI GC/CIMS NH3/CH4G 145/270 A/D=3

m/z	abund.	m/z	abund.	m/z	abund.	m/z	abund.
154.15	1000	182.20	27	230.95	3	261.20	631
155.15	139	184.30	19	243.20	38	262.30	157
156.15	571	194.30	6	244.20	136	263.20	699
157.15	58	196.30	5	245.20	82	264.20	142
156.25	5	228.05	18	246.20	138	265.20	35
168.15	6	229.30	4	247.05	55	265.95	6
170.25	3	230.05	15	248.20	10	266.20	8
173.00	4						

SUBSTITUTE SHEET (RULE 26)



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/04683

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :G01N 33/53

US CL :436/518; 435/7.1

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/518, 501, 528, 531; 435/7.1; 530/333, 334; 536/18.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN:

search terms: combinatorial, library, tag, identifier, reaction history

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	PROCEEDING OF THE NATIONAL ACADEMY OF SCIENCES USA, VOLUME 90, ISSUED DECEMBER 1993, OHLMEYER ET AL., "COMPLEX SYNTHETIC CHEMICAL LIBRARIES INDEXED WITH MOLECULAR TAGS", PAGES 10922-10926, SEE ENTIRE ARTICLE.	1-15, 20-37, 45, 50-52 ----- 16-19, 38-44, 46-49, 53-84
Y	TRENDS IN ANALYTICAL CHEMISTRY, VOLUME 2, NUMBER 7, ISSUED 1983, GIESE, "ELECTROPHORIC RELEASE TAGS: ULTRASENSITIVE MOLECULAR LABELS PROVIDING MULTIPLICITY", PAGES 166-168. SEE ENTIRE DOCUMENT.	16-19, 38-40, 46-49, 53-84
Y	US, A, 4,755,558 (KALBAG) 05 JULY 1988, COLUMNS 6- 9.	16-19, 38-40, 46-49, 53-84

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"Z"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

24 JUNE 1995

Date of mailing of the international search report

14 JUL 1995

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

LORA M. GREEN

Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)\*

